

A BUFFER FOR A CAS PROTEIN AND A METHOD OF USING THEREOF**FIELD OF THE INVENTION**

[0001] The present invention belongs to the field of buffer for Cas protein to enhance
5 its enzymatic cleavage activity. The present invention also relates to the field of nucleic
acid detection by CRISPR-Cas using said buffer.

BACKGROUND

[0002] Recently novel Cas13 enzymes which can be used in clustered regularly
10 interspaced short palindromic repeats (CRISPR/Cas)-based methods for detecting nucleic
acids were disclosed in PCT/SG2022/050859. This approach has gained prominence as
viable alternatives to traditional methodologies. These approaches are characterized by
their independence from specialized instrumentation, alongside their simplicity,
sensitivity, specificity, affordability, and rapidity. Additionally, they possess the capacity
15 for multiplex pathogen detection within a single assay. The novel aspect of these
methodologies lies in leveraging the collateral cleavage activity of Cas proteins,
facilitating the transformation of target nucleic acid sequences into observable signals.
When a Cas protein such as Cas13, in complex with CRISPR RNA (crRNA), identifies
its target RNA, it becomes activated. This activation triggers Cas13 to indiscriminately
20 cut the quenched fluorescent ssRNA reporter molecules. As a result of this cleavage, the
fluorescent dye is released, producing a detectable signal.

[0003] There is a need to understand how the efficiency of enzymatic cleavage by
Cas13 can be influenced by a variety of reaction buffer conditions, in order to find an
optimal buffer composition, and optimize the trans-cleavage activities of Cas13. To refine
25 the Cas13 reaction buffer, various commercially available buffers were initially evaluated
to define the best buffer for Cas13 trans-cleavage activity. Several published or
commercially available buffer systems that are marketed or commonly used for Cas13-
based diagnostics, including buffers from IDT (used in SHERLOCK assays) and other
vendors offering CRISPR diagnostic kits, were evaluated. These buffers were primarily
30 optimized for earlier and well-characterized Cas13 enzymes such as LwaCas13a or
LbuCas13a. However, these commercial buffers often did not support optimal activity for
the inventors' novel Cas13 enzymes. Specifically, they resulted in reduced collateral

cleavage efficiency, delayed reaction kinetics, and lower overall signal intensity. This highlighted the need for a customized buffer formulation, as commercial solutions may not be universally compatible with newly developed or less-characterized Cas13 variants. Enzymatic cleavage activities are widely recognized to be influenced by numerous reaction conditions such as ion types, salt concentrations, and other factors. Consequently, a thorough investigation of the variables impacting the trans-cleavage activities of Cas13 was conducted. By systematically analysing and optimizing these reaction conditions, the optimal buffer that maximizes Cas protein activity, such as Cas13 activity was able to be identified and established.

SUMMARY

[0004] In one aspect, the present disclosure refers to a buffer for enhancing enzymatic cleavage activity of a Cas protein, comprising one or more components selected from the group consisting of:

a salt selected from the group consisting of monopotassium phosphate (KH_2PO_4), sodium formate (HCOONa), calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$), sodium acetate (CH_3COONa), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), potassium thiocyanate (KSCN), calcium chloride (CaCl_2), sodium chloride (NaCl), Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$), lithium sulfate (LiSO_4);

an organic solvent selected from the group consisting of Tween 20, Dithiothreitol (DTT),

Phenylmethylsulfonyl fluoride (PMSF), 2-Methyl-2,4-pentanediol (MPD), acetone, dimethylsulfoxide (DMSO), ethylene glycol, methanol, 2-propanol, EDTA, urea; and

a protein which is casein.

[0005] In another aspect, the present disclosure refers to a detection kit for a nucleic acid, comprising:

(a) the buffer as disclosed herein;

(b) a Cas protein selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c, Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f; and

(c) a crRNA configured to target the nucleic acid and form a complex with the Cas protein.

[0006] In another aspect, the present disclosure refers to a method of detecting a nucleic acid in a sample by clustered regularly interspaced short palindromic repeats and

CRISPR associated protein (CRISPR-cas), using the detection kit as disclosed herein, the method comprising: incubating the sample containing the nucleic acid with the Cas protein and the crRNA, wherein when the nucleic acid is present in the sample, enzymatic cleavage activity of the Cas protein is induced to generate a detectable signal when the probe nucleic acid is cleaved; wherein the buffer enhances the enzymatic cleavage activity of the Cas protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0008] **Figure 1** shows ionic influence on Cas13-Cd14 activity under varying salt conditions. a. the influence of KH_2PO_4 , CdSO_4 , $\text{Co}(\text{NO}_3)_2$, HCOONa , $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$, CH_3COONa , b. the influence of $(\text{NH}_4)_2\text{SO}_4$, AlCl_3 , KSCN , FeSO_4 , CuSO_4 , LiSO_4 , c. the influence of $\text{Al}_2(\text{SO}_4)_3$, ZnCl_2 , CaCl_2 , NaCl , K_2SO_4 . The fluorescence signal measured after 5 min has been presented here. Values are shown in the graphs as means $\pm$ SD ($n = 3$). The dotted line in the graph represents the baseline activity, as determined by the control reaction buffer.

[0009] **Figure 2** shows the influence of organic compounds on Cas13-Cd14 activity. a. the influence of DTT, urea, EDTA and PMSF. b. the influence of ethylene glycol, methanol, DMSO, Tween 20, 2-Propanol, acetone, and MPD. The fluorescence signal measured after 5 min has been presented here. Data are represented as mean values with standard deviation (mean $\pm$ SD, $n = 3$).

[0010] **Figure 3** shows the effect of casein on the collateral cleavage activity of Cas13-Cd14. The fluorescence signal measured after 5 min has been presented here. Values are shown in the graphs as means $\pm$ SD ($n = 3$).

[0011] **Figure 4** shows ionic influence on Cas13-Cd14 activity in SARS-CoV-2 detection. a. the influence of LiSO_4 and $\text{Al}_2(\text{SO}_4)_3$. b. the influence of $\text{Ni}(\text{NO}_3)_2$ and ZnCl_2 c. the influence of CaCl_2 and NaCl . The fluorescence signal measured after 5 min has been presented here. Values are shown in the graphs as means $\pm$ SD ($n = 3$).

[0012] **Figure 5** shows the influence of organic compounds on Cas13-Cd14 activity. a. the influence of urea and PMSF. b. the influence of EDTA and DMSO. c. the influence of Tween 20 and acetone. d. the influence of MPD and methanol. e. the influence of propanol and ethylene glycol. The fluorescence signal measured after 5 min has been presented here. Data are represented as mean values with standard deviation (mean $\pm$ SD, n = 3).

[0013] **Figure 6** shows the impact of Casein on Cas13-Cd14 activity in SARS-CoV-2 detection. The fluorescence signal measured after 5 min has been presented here. Values are shown in the graphs as means $\pm$ SD (n = 3).

[0014] **Figure 7** shows real-time fluorescence signals of Cas13-Cd14 at varying SARS-CoV-2 DNA concentrations. Real-time PCR signals were monitored different target DNA concentrations (0.0015, 0.0075, 0.015, 0.015, and 1.5 copies/ μ L). The fluorescence was measured in 1 min intervals for a total of 60 min. The measurement of real-time background subtracted fluorescence output are shown in the graphs as means $\pm$ SD (n = 3).

[0015] **Figure 8** shows enhancement of Cas13-Cd13 and Cas13-Cd15 activity by Tween 20, MPD, and Casein. Fluorescence intensity was measured to assess the collateral cleavage activity of Cas13-Cd13 and Cas13-Cd15 in the presence of Tween 20, MPD, and Casein. The fold increase in fluorescence signal relative to the no-additive control is shown. Tween 20 and Casein exhibited the highest enhancement effect on Cas13-Cd15, increasing activity by 10.4-fold and 11.2-fold, respectively. Data represent the mean $\pm$ standard deviation (n = 3).

DETAILED DESCRIPTION

[0016] To enhance enzymatic efficiency of a Cas enzyme such as Cas13 and Cas12 for collateral cleavage and detection of nucleic acids, such as nucleic acids of pathogens, novel compounds (salts, organic solvents and proteins) were introduced into the enzyme reaction buffer, presenting a remarkable increase in enzyme collateral cleavage activity.

[0017] In one aspect, the present disclosure refers to a buffer for enhancing enzymatic cleavage activity of a Cas protein, comprising one or more components selected from the group consisting of:

a salt selected from the group consisting of monopotassium phosphate (KH_2PO_4), sodium formate (HCOONa), calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$), sodium acetate (CH_3COONa), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), potassium thiocyanate (KSCN), calcium chloride (CaCl_2), sodium chloride (NaCl), Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$), lithium sulfate (LiSO_4);

- 5 an organic solvent selected from the group consisting of Tween 20, Dithiothreitol (DTT), Phenylmethylsulfonyl fluoride (PMSF), 2-Methyl-2,4-pentanediol (MPD), acetone, dimethylsulfoxide (DMSO), ethylene glycol, methanol, 2-propanol, EDTA, urea; and a protein which is casein.

[0018] The technical aspects of the present invention that distinguish it from existing
10 technologies include the precise selection and addition of above-mentioned components for the first time to the Cas enzyme (such as Cas13) reaction buffer. Each of these compounds has been individually proven to enhance collateral cleavage activity of the Cas enzyme (such as Cas13), and therefore, its capability for target nucleic acid detection, for example pathogen detection. The unique features and benefits of the present invention
15 in comparison to existing technologies are:

- **Enhanced Enzyme Activity:** The individual addition of each compound has resulted in a substantial increase in the Cas enzyme (such as Cas13 and Cas12) collateral cleavage activity, demonstrating the present buffer composition's effectiveness.
- 20 • **Customized Buffer Composition:** The deliberate choice and ratio of salts, solvents, and protein in the buffer are tailored to not only augment the Cas enzymatic activity but also to ensure the stability and efficiency of the Cas enzyme across a broader range of conditions.
- **Versatility in Application:** The enhanced activity of the Cas enzyme (such as
25 Cas13 and Cas12) by the present compounds expands the potential applications, particularly in the field of pathogen detection, where rapid and efficient detection of pathogen can be critical.
- **Increased Sensitivity for Pathogen Detection:** The higher activity levels obtained could lead to increased sensitivity in diagnostic tests that rely on the Cas enzyme
30 (such as Cas13 and Cas12) collateral cleavage activity, enabling the detection of pathogens at lower concentrations than currently possible.

- **Cost-Effectiveness:** By increasing the specific activity of the Cas enzyme (such as Cas13 and Cas12), less enzyme may be required for the same or enhanced effect, leading to reduced costs for industrial-scale applications.
- 5 • **Adaptability:** The improved activity under different conditions suggests that the Cas enzyme (such as Cas13 and Cas12) could be adapted for use in a variety of environments, which is particularly beneficial for field-based pathogen detection where laboratory conditions cannot be replicated.
- 10 • **Synergistic effects of the buffer components:** The synergistic combination of these compounds, which have individually shown to amplify activity, yields a cumulative increase potentially exceeding current level of activity, enhancing enzymatic efficiency significantly.
- 15 • **Broader applicability to a variety of Cas enzymes:** The disclosed compounds which enhance the activity of Cas13-Cd13, Cd14 and Cd15 enzymes. While the present experimental data demonstrate enhancement of Cas13 enzymatic activity through the addition of novel buffer components, it can be reasonably anticipated that similar improvements may extend to Cas12 enzymes. This expectation is based on several shared mechanistic and structural features between Cas13 and Cas12 systems. Both enzymes are RNA-guided nucleases that require precise buffer conditions to maintain optimal tertiary conformation and catalytic activity.
- 20 Furthermore, the identified compounds act through mechanisms such as stabilizing protein-RNA interactions, enhancing target binding, or facilitating ion-dependent conformational changes—features that are also critical for Cas12 function. Given these mechanistic similarities, it is plausible that these novel buffer additives will likewise enhance Cas12 activity. This potential cross-
- 25 applicability supports the broader utility of the present buffer system across the CRISPR-Cas enzyme family.

[0019] In general, the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful tool for gene editing that allows scientists to precisely alter DNA sequences and modify gene function. It was originally discovered as a natural defense mechanism in bacteria and archaea, where it helps these organisms fend off viral infections. CRISPR works via the following steps: (1) Recognition: When a virus invades a bacterial cell, the bacterium incorporates a segment of the viral genetic material—

typically DNA, but in some cases RNA (converted to DNA by reverse transcription)—into its own genome at the CRISPR locus. This segment is known as a "spacer." (2)

Transcription: The CRISPR locus is transcribed into a long RNA molecule, which is then processed into shorter "guide RNAs" (gRNAs) that include sequences complementary to

5 the viral DNA or RNA. (3) Targeting: The guide RNA forms a complex with a CRISPR-associated (Cas) protein. This complex can recognize and bind to the corresponding viral DNA and RNA sequence. (4) Cleavage: The Cas protein acts as a molecular scissor, cutting the viral DNA and RNA at the targeted location, thereby neutralizing the threat.

Applications of CRISPR system includes: (1) Gene Editing: CRISPR can be used to add,

10 remove, or alter specific DNA and RNA sequences in the genome of living organisms.

This has vast implications for research, medicine, and agriculture. (2) Gene Therapy: CRISPR is being explored as a potential treatment for genetic disorders by correcting mutations at the DNA level. (3) Functional Genomics: Researchers use CRISPR to study gene function by creating targeted mutations and observing the resulting phenotypes. In

15 the context of the present disclosure, CRISPR is used to detect target nucleic acid of a particular pathogen for pathogen or disease detection.

[0020] Cas13 is an RNA-targeting enzyme that belongs to the CRISPR family, specifically the Type VI CRISPR-Cas systems. It is comprised of subtypes VI-A, VI-B, VI-C, and VI-D and also known as C2c2 or CasRx (type VI-D). Unlike the well-known

20 Cas9, which targets DNA, Cas13 specializes in targeting and cleaving single-stranded RNA. This makes it a powerful tool for RNA manipulation and has opened up exciting possibilities in biotechnology and medicine. Cas13 enzymes are classified into several subtypes based on their structural and functional differences. The diverse Cas13 family contains at least four known subtypes, including Cas13a (formerly C2c2), Cas13b, 25 Cas13c, and Cas13d. All known Cas13 family members contain two HEPN domains which confer RNase activity. Cas13s function similarly to Cas9, using a ~64-nt guide RNA to encode target specificity. The Cas13 protein complexes with the guide RNA via recognition of a short hairpin in the crRNA, and target specificity is encoded by a 28 – 30-nt spacer that is complementary to the target region.

30 [0021] The CRISPR-Cas13 system has a target-specific nucleic acid cleavage activity, and two conditions are required to exhibit this target-specific nucleic acid cleavage activity: (1) Recognition of a Specific Nucleotide Sequence: The Cas13 protein must

recognize a nucleotide sequence of a certain length in the nucleic acid. (2) Complementary Binding of the Guide RNA: The guide RNA (crRNA) must have a sequence that can complementarily bind to the target sequence around the recognized nucleotide sequence. This binding ensures that the Cas13 protein is directed to the correct location on the nucleic acid. When the above two conditions are satisfied, 1) the Cas13 protein recognizes the nucleotide sequence of a certain length, and 2) the guide RNA complementarily binds to a portion of the sequence surrounding the nucleotide sequence of the certain length, nucleic acid cleavage activity is exhibited.

[0022] In addition to programmable RNase activity, all Cas13s exhibit collateral cleavage activity after recognition and cleavage of a target RNA, leading to non-specific degradation of any nearby single-stranded RNA molecules regardless of complementarity to the spacer. Properties of Cas13 make it well suited for nucleic acid detection. When the enzyme recognizes its target *in vitro*, it becomes activated and promiscuously cleaves RNA species in solution. Cas13-based detection is specific, and can be tuned for single-nucleotide distinction at any position on the target. This secondary cleavage activity can be utilized to detect the presence of a target nucleic acid in a sample, making the CRISPR-Cas13 system a powerful tool for diagnostic applications.

[0023] Cas12, also known as Cpf1 (type V-A) or C2c1 (type V-B), is a CRISPR-associated protein that belongs to the Type V CRISPR-Cas systems. It is an RNA-guided endonuclease that targets and cleaves double-stranded DNA. Cas12 is a compact and efficient enzyme that creates staggered cuts in dsDNA. Cas12 processes its own guide RNAs, leading to increased multiplexing ability. Cas12 has also been engineered as a platform for epigenome editing, and it was recently discovered that Cas12a can indiscriminately chop up single-stranded DNA once activated by a target DNA molecule matching its spacer sequence. This property makes Cas12a a powerful tool for detecting tiny amounts of target DNA in a mixture. The Cas12 protein requires only the crRNAs to create an efficient cut at ssDNA and dsDNA. Cas12 protein contains the RuvC and nuclease lobe (NUC) domains for cleavage activity. Like Cas9, Cas12 encounters a potential target site beside a PAM sequence. Once Cas12 starts encountering, it initiates R-loop, which forms base-pair hybridization between the crRNA and the target DNA strand. During this step, Cas12 matches the <17 bp of the target sequence and leads to an R-loop formation. Once R-loop is formed, the Cas12 protein uses its active RuvC domain

to cleave the non-target strand with the help of the PAM sequence. Cas12 has been widely used in genome editing, diagnostics (like the DETECTR system for detecting pathogens), and other biotechnological applications. Subtypes of Cas12 enzymes include Cas12a (Cpf1) known for creating staggered cuts (sticky ends) in DNA, making it useful for
5 precise genome editing. Other subtypes of Cas12 include Cas12b, Cas12c, Cas12d, Cas12e and Cas12f. The CRISPR-Cas12 system has a collateral cleavage activity in addition to the target-specific nucleic acid cleavage activity. When the CRISPR-Cas12 system recognizes and binds to a target nucleic acid, it activates the Cas12 protein, which then indiscriminately cleaves single-stranded nucleic acids in the vicinity. This secondary
10 cleavage activity can be utilized to detect the presence of a target nucleic acid in a sample, making the CRISPR-Cas12a system a powerful tool for diagnostic applications.

[0024] CRISPR detection is generally divided into 2 parts. The first step is usually the amplification of a target sequence, which specifically comprises the steps of amplifying target nucleic acid by utilizing the technologies of PCR, RT-PCR, RPA, LAMP and the
15 like; the second step is a trans-cleavage reaction of the Cas protein: adding a reporter, such as a non-specific single-stranded DNA or RNA reporter (ssDNA-reporter or ssRNA-reporter) with a fluorescent emitting group at one end and a fluorescent quenching group at one end into the system, and exciting the cleavage activity of the single-stranded DNA or RNA fluorescent reporter after the Cas protein-crRNA complex is combined with the
20 target DNA, thereby generating a free fluorescent emitting group and emitting detectable fluorescence. In addition to this, CRISPR-Cas detection can be performed in a one-pot system, where target amplification, CRISPR-based recognition, and signal detection occur within a single reaction vessel without the need for multiple handling steps.

[0025] To enhance the sensitivity and efficiency of the target nucleic acid detection, superior trans-cleavage activity of the Cas protein is desired, and this can be achieved or
25 aided by the buffer disclosed herein.

[0026] The present disclosure discloses a buffer for enhancing enzymatic cleavage activity of a Cas protein. In one example, the Cas protein is a Cas13 protein selected from the group consisting of Cas13d, Cas13a, Cas13b, and Cas13c. In another example, the
30 Cas protein is a Cas12 protein selected from the group consisting of Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f. The buffer has wide applications to enhance enzymatic

cleavage activity of various Cas proteins as disclosed herein which possess collateral cleavage activity.

[0027] As used herein, Cd13, Cas13-Cd13, or Cas13d-Cd13 are interchangeable terms. As used herein, Cd14, Cas13-Cd14, or Cas13d-Cd14 are interchangeable terms.

5 As used herein, Cd15, Cas13-Cd15, or Cas13d-Cd15 are interchangeable terms. Cd13 Cd14, Cd15 refer to Cas13 protein variants. All of these Cas13 variants can be used in the context of CRISPR technology for precise genome editing, diagnostics, and other biotechnological applications.

[0028] In one example, the Cas13 protein is Cas13-Cd13, which has an amino acid
10 sequence of SEQ ID NO: 2 as shown in Table 1:

[0029] VSKNDNIKSKAKALGLKSTFQVGDEVVMTSFGKGNKAIVEKIVRGTDVQSVPTENFSAEIEGKKFDLVGR
AHIQTKSDNPQYSKKRTGDDMIGAKAALEKRFGGTFDDNIHQLAYNVLDIEKILSVHINNIVYTLNNIRRKDNAEDDDFIG
YMSTRNDYDTFIEPRKHNISEDAKSIDKSASFEEYLQPPVKDCLHYFGNTFFAPREIEKTYTDNRGFERKKKVTVNSLI
DEKEIYYIFALLGGLRQFCTHDNKSGRNWLYSLEENGINSDAKAVLDKYYNSAVARIDESFVDNASKTNFKLIFNAMDVSD
15 EALQDNIAKAFYKFTVCKSFKNMGFSIKKLREQLLEPEYGLKDKHYDSVRSKLYQIMDFVIYLSFKDEKFKKDNEEKINN
IVNELRATLNEDKGRVYASYAERMKSELKPAIARLKSIDIKDSRVKEFELDASVKYRLSKVSVRLKDRATYFTKLIY
LTTFLDGKEINDLLTTLHQFENIASFDVMNDRGIDCRFSDGYKLFESSKQIAFELRNVSFARMTRTSKDDENATHMMY
IDAAEILGTDYTEEQIEEHLNLEKKRMIPGTTKADMNFRNFIINNVIKSSRFNYLVRYSNPKKIRALADNEGVIKFLGELPD
AQIDRYTLGCFNPDADROEKTDKLAKAITGLRFNDFENVKQGANTEGESQESIDKAQKQGLISLYLTVLYLLTKNLVYYN
20 SRYFLAFHCLERDAQLLGSGAGHHEPYVALTQRFINEKLNHACEYKLTNIANSDEYTIIRFRNNAHLSAVRNANLYID
KLKEFKSYIEYHFLSQENIYKGYCVDKKYVTADENGSKTISVKISKDYCPQVYIDKSLEYFDKLNKYGTICKDFTKALNSP
FGYNLARYKNLSIEGLFDRNRPGDKGENTFED

[0030] In another example, the Cas13 protein is Cas13-Cd14, which has an amino acid
sequence of SEQ ID NO: 4 as shown in Table 1:

[0031] MAKKLSPKEIREAAKAEKMSIKAAEAEREKAAEEAKLKAEEKKEKAEKNEREKALKRFRLEKSRMAL
PKSERKSLAKAAGVKSFAVGNDIYLTSTFDRGNDIAVEKITDTVTNLRSDSEFVENNTITEMSVPIKSKRISLDYAIADN
PLYRKDSATKVQPDKLLKDTLEKLYFGKTFDDTLHIQIYNILDEKILTVYSINTIYCLNNLFGKESGEKEDLISKLTQITYD
EFKESKAHNEFIDFYNLNTLGGYGNIFFEKKEKRSQKEIYDIIALITIRQWCVHCEEDKRTWLFNTETVLSKEFLDILDVY
ESLVEKVNRFNFKDNKVNLIQILEDVLEIKDSERELIRQYYRFIVTKEQKLLGFSIKKLREAMELETFKTDKKYDTVRSK
30 LYKLIDFLFTGYTTDEAEKEKALFLIKSLRESLREETKDRIYKSEARLWLKYENTITNKIREALNEKSISELKKDKSFDDKSI
TSITDEVSGKKATYFSKTIYLLAQFIDGKEVNDLTTSLINKFDNIRSLIDTAGQIGLDCKFTEEYKFFENSQDQIRTELHVIKNL
TNMEYYDDTVKKQMYKDAVHILGIQDDVSDAELEKIINSILLNENGKPLPGTKGKKGFRNFIISNVLSRRFIYLIKYNPK
KIRKIAGNRKIIKVLRSRITDSQLERYYYSCNPELKSIGYPRDDAVNDLSILIADMKFEDFKNVDQSANVHDNNAAAREKM
KYQTIISLYLTVCYHLVKNLVNINARVAMAFHALERDARLYQIFSSEENYVDNLNADYAILTKTLLKDNENAGNLYLRNKK
35 WNKLTRENLDNYPQAAANFRNAVHNLPIRNADMLLEDIEDVSSYYAIYHYIMQKSVTNRTIRVSNNTTEDEKRLTDYQN
KIKRHHGYNKDFVKALCVFAYNIVRFKLSIYEMFDRNYHEKTSPEITDSDSQSP

[0032] In another example, the Cas13 protein is Cas13-Cd15, which has an amino acid
sequence of SEQ ID NO: 6 as shown in Table 1:

[0033] MDKEKTTVEGKNTNQKSDVLKSLAKANGLKSSFVIGNEVVMTSFGRGNSAILEKKITGSRIENLNPNVAFY
40 VKKHISDSCNPDSKGYDVKSKRMKEKAVVDDPVYVSPEKASNVHAGQDLIGCKNVLEERYFGKTFDDNIHQILIYNILDE
KILAVHVSNAFAINNIGIEGKENEDFIGNLSVLNTFDEFENYETHPKFANKSAIKENLRKSKVFFDKIKKGNKLGYPGQAF

YYATGTGKNLIFTKKSSSETIYELLALVGSRLQFCVHDEVMDNKKVKSRSWLYNAQKELKPDFLKALDELYSKEVEKIDSDFI
 IVNNTVDLHIIHDAIDVIDGSADWQKITNEYDYDFIIRKCFKNIGFSIKRLRETMIEEQMKVLGKCDRENCKGYGKCFKNKKY
 DSVRSRLNRIVDFIIFRHYNDEAILKNVSLRLTCMSEEEKQKRFYLPEAKALWKYNYVFRNYVLKKLNGKSISGLKEKAIE
 NSIDINSYKISLGDYPDYFCKFIYLLTFFLDGKEINDLLTTLINKFDNIASFISVMKNDKLSIDCEFPVEYSFFANSAQITSDLRVI
 5 NSFARMQAPAEPSKDDMYRDALDILGMDLSEDGKKQLEDTVLCRDENGKYMKKEDNNPKRDTNFRNFLGNVLA
 RFKYLIRYNNNAKTRALANNKAVIIFMLNKINKQNPEQIVSYKACRDDSDPVASDAEAKIEFLAEKIMNVSCQFRYVKNG
 TKVRPDEAKEKERFKAIIGLYLTVMYLITKNMVIYNSRYVTAHCLERDSELHGKFDQKKLQPNLTCKFIDPKTCGDYGL
 RNNKRARTYIEQNMDKMSNCTSYWNEYRNAVAHLSVIRNMNQYIKNVKNIGSCFELYHYIMQRFLLDKENIAESLREYDD
 FIKKKGCYRKDFVKALNTPFGYNLARYKNLSIAELFDRNDELTENKLRNEAIKADIEEI

10 [0034] The buffer as disclosed herein can not only enhance cleavage activity of the
 Cas13 protein variants disclosed herein, but also can enhance other Cas protein of the
 same or similar clade. As used herein, the term "clade" refers to a group of organisms (or
 genes or proteins, in this case) that have evolved from a common ancestor. Specifically,
 it means that the Cas13 or Cas12 proteins as disclosed herein and any other Cas13 or
 15 Cas12 proteins within the same clade share a common evolutionary origin and thus have
 similar genetic and structural characteristics.

[0035] The DNA sequence encoding SEQ ID NO: 2 is SEQ ID NO: 1. The DNA
 sequence encoding SEQ ID NO: 4 is SEQ ID NO: 3. The DNA sequence encoding SEQ
 ID NO: 6 is SEQ ID NO: 5. These DNA and amino acid sequences are shown in **Table**
 20 **1**.

[0036] As used herein, the terms "polypeptide", "peptide", and "protein" used herein
 are used interchangeably in this application to refer to a polymer of amino acid residues.
 The term applies to amino acid polymers in which one or more amino acid residues are
 an artificial chemical analog of the corresponding naturally occurring amino acid, and to
 25 naturally occurring amino acid polymers. The terms "polypeptide", "peptide", "amino
 acid sequence" and "protein" may also include modified forms including, but not limited
 to, glycosylation, lipid linkage, sulfation, gamma carboxylation of glutamic acid residues,
 hydroxylation and ADP-ribosylation. Unless otherwise stated, amino acid sequences
 disclosed herein are written in the N-terminal to C-terminal direction using one-letter
 30 notation. For example, when expressed as RNVP, it means a peptide in which arginine,
 asparagine, valine, and proline are sequentially connected from the N-terminal to the C-
 terminal. The method of notation of each amino acid is as follows: Alanine (Ala, A);
 Arginine (Arg, R); Asparagine (Asn, N); Aspartic acid (Asp, D); Cysteine (Cys, C);
 Glutamic acid (Glu, E); Glutamine (Gln, Q); Glycine (Gly, G); Histidine (His, H);
 35 Isoleucine (Ile, I); Leucine (Leu, L); Lysine (Lys, K); Methionine (Met, M); Phenylalanine

(Phe, F); Proline (Pro, P); Serine (Ser, S); Threonine (Thr, T); Tryptophan (Trp, W); Tyrosine (Tyrosine; Tyr, Y); and Valine (Val, V).

[0037] As used herein, the terms "polynucleotide", "nucleic acid sequence", "nucleotide sequence" or "nucleic acid fragment" are used interchangeably and are single-
5 or double-stranded RNA or DNA polymers, optionally containing synthetic unnatural, or altered nucleotide bases. Nucleotides are referred to by their single letter designations as follows: "A" for adenosine or deoxyadenosine (for RNA or DNA, respectively), "C" for cytidine or deoxycytidine, "G" for guanosine or deoxyguanosine, "U" means uridine, "T" means deoxythymidine.

10 [0038] The buffer as disclosed herein comprises a base composition comprising HEPES, KCl, MgCl₂, and glycerol. A base composition means that these components in the composition must be present in all embodiments of the buffer disclosed in this invention.

[0039] In one example, the base composition is one comprising about 20 mM HEPES,
15 about 50 mM KCl, about 5 mM MgCl₂, about 5% glycerol, and having a pH of about 6.8;

[0040] In another example, the base composition is one comprising about 20 mM HEPES, about 50 mM NaCl, about 10 mM MgCl₂, about 5% glycerol, and having a pH of about 7.0;

[0041] In another example, the base composition is one comprising about 20 mM
20 HEPES, about 50 mM NaCl, about 10 mM MgCl₂, about 5% glycerol, about 1 µg/ml BSA, and having a pH of about 7.0;

[0042] In another example, the base composition is one comprising about 20 mM HEPES, about 60 mM NaCl, about 6 mM MgCl₂, about 5% glycerol, and having a pH of about 6.8;

25 [0043] In another example, the base composition is one comprising about 20 mM HEPES, about 60 mM NaCl, about 5% PEG, about 5 µM DTT, and having a pH of about 6.8; and

[0044] In another example, the base composition is one comprising about 20 mM HEPES, about 60 mM KCl, about 5% PEG, and having a pH of about pH 8.0.

30 [0045] The buffer as disclosed herein further comprises one or more components selected from the group consisting of a salt, an organic solvent, and a protein.

[0046] The salt of the buffer as disclosed herein is selected from the group consisting of monopotassium phosphate (KH_2PO_4), sodium formate (HCOONa), calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$), sodium acetate (CH_3COONa), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), potassium thiocyanate (KSCN), calcium chloride (CaCl_2), sodium chloride (NaCl), Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$), lithium sulfate (LiSO_4).

[0047] In one example, the concentration of each of the salt as disclosed herein is 5 mM-3 M, 10-60 mM, or 0.25-10 mM. In some examples, the concentration of each of the salt as disclosed herein is 0.25-10 mM, 0.25-0.5 mM, 0.5-1 mM, 1-2.5 mM, 2.5-5 mM, 5-10 mM, 0.25-9.5 mM, 1-9 mM, 1.5-8.5 mM, 2-8 mM, 3.5-7.5 mM, 4-7 mM, 4.5-6.5 mM, or 5-6 mM. In some examples, the concentration of each of the salt as disclosed herein is 10-60 mM, 10-20 mM, 20-30 mM, 30-40 mM, 40-50 mM, 50-60 mM, 20-50 mM, or 30-40 mM. In some examples, the concentration of each of the salt as disclosed herein is 5 mM-3M, 10 mM – 2 M, 20 mM – 1 M, 30 mM – 900 mM, 40 mM – 800 mM, 50 mM – 700 mM, 100-600 mM, 120 mM – 500 mM, 150 mM – 400 mM, 200 mM -300 mM, 5-15 mM, 10-20 mM, 15-30 mM, 20-40 mM, 30-50 mM, 40-100 mM, 50-200 mM, 100-300 mM, 200-400 mM, 300-500 mM, 400-600 mM, 500-700 mM, 600-800 mM, 700-900 mM, 800 mM-1M, 1-3M, or 1.5-2 M. In some examples, the concentration of each of the salt as disclosed herein is about 0.25 mM, about 0.5 mM, about 1 mM, about 2.5 mM, about 5 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, about 1 M, about 1.5 M, about 2 M, about 2.5 M, or about 3M.

[0048] In one example, the salt is monopotassium phosphate (KH_2PO_4) and the concentration of monopotassium phosphate (KH_2PO_4) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0049] In another example, the salt is sodium formate (HCOONa) and the concentration of sodium formate (HCOONa) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0050] In another example, the salt is calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$) and the concentration of calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$) is 10-60 mM, for example 10-30 mM,

20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0051] In another example, the salt is sodium acetate (CH_3COONa) and the concentration of sodium acetate (CH_3COONa) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0052] In another example, the salt is ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) and the concentration of ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0053] In another example, the salt is potassium thiocyanate (KSCN) and the concentration of potassium thiocyanate (KSCN) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM.

[0054] In another example, the salt is calcium chloride (CaCl_2) and the concentration of calcium chloride (CaCl_2) is 10-60 mM, for example 10-30 mM, 20-40 mM, 30-50 mM, 40-60 mM, 20-50 mM, 30-40 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, or about 60 mM; or 1.25-50 mM, for example 1.25-10 mM, 2.5-5 mM, 5-12.5 mM, 12.5-15 mM, 10-20 mM, 15-25 mM, 20-30 mM, 25-35 mM, 30-40 mM, 35-45 mM, 40-50 mM, 2.5-45 mM, 5-40 mM, 10-35 mM, 12.5-30 mM, about 1.25 mM, about 2.5 mM, about 5 mM, about 10 mM, about 12.5 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, or about 50 mM.

[0055] In another example, the salt is sodium chloride (NaCl) and the concentration of sodium chloride (NaCl) is 0.25-10 mM, for example 0.25-1 mM, 0.5-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or 1.25-50 mM, for example 1.25-10 mM, 2.5-5 mM, 5-12.5 mM, 12.5-15 mM, 10-20 mM, 15-25 mM, 20-30 mM, 25-35 mM, 30-40 mM, 35-45 mM, 40-50 mM, 2.5-45 mM, 5-40 mM, 10-35 mM, 12.5-30 mM, about

1.25 mM, about 2.5 mM, about 5 mM, about 10 mM, about 12.5 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, or about 50 mM.

[0056] In another example, the salt is Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$) and the concentration of Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$) is 0.25-10 mM, for example 0.25-1 mM, 0.5-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM.

[0057] In another example, the salt is lithium sulfate (LiSO_4) and the concentration of lithium sulfate (LiSO_4) is 0.25-10 mM, for example 0.25-1 mM, 0.5-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM.

[0058] The organic solvent of the buffer is selected from the group consisting of Tween 20, Dithiothreitol (DTT), Phenylmethylsulfonyl fluoride (PMSF), 2-Methyl-2,4-pentanediol (MPD), acetone, dimethylsulfoxide (DMSO), ethylene glycol, methanol, 2-propanol, EDTA, urea.

[0059] In one example, the concentration of each of the organic solvents disclosed herein is 5 mM - 3 M, or 1-5 mM. In some examples, the concentration of each of the organic solvents as disclosed herein is 1-5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 1.5-4.5 mM, 2-4 mM. In some examples, the concentration of each of the organic solvents as disclosed herein is 5 mM-3M, 10 mM - 2 M, 20 mM - 1 M, 30 mM - 900 mM, 40 mM - 800 mM, 50 mM - 700 mM, 100-600 mM, 120 mM - 500 mM, 150 mM - 400 mM, 200 mM - 300 mM, 5-15 mM, 10-20 mM, 15-30 mM, 20-40 mM, 30-50 mM, 40-100 mM, 50-200 mM, 100-300 mM, 200-400 mM, 300-500 mM, 400-600 mM, 500-700 mM, 600-800 mM, 700-900 mM, 800 mM-1M, 1-

3M, or 1.5-2 M. In some examples, the concentration of each of the organic solvents as disclosed herein is about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, about 1 M, about 1.5 M, about 2 M, about 2.5 M, or about 3M.

[0060] In one example, the organic solvent is DTT and the concentration of DTT is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 1-5 mM, for example 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 1-4 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, or about 5 mM.

[0061] In another example, the organic solvent is PMSF and the concentration of PMSF is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or 1-2.5 mM, for example 1-2 mM, 1.5-2.5 mM, about 1 mM, about 1.5 mM, about 2 mM, or about 2.5 mM.

[0062] In another example, the organic solvent is DMSO and the concentration of DMSO is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7

mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or 1-2.5 mM, for example 1-2 mM, 1.5-2.5 mM, about 1 mM, about 1.5 mM, about 2 mM, or about 2.5 mM.

[0063] In another example, the organic solvent is Tween 20 and the concentration of Tween 20 is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or about 2.5-5 mM, for example 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 2.5-4 mM, 3-3.5 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, or about 5 mM.

[0064] In another example, the organic solvent is ethylene glycol and the concentration of ethylene glycol is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about

0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or about 2.5 mM.

[0065] In another example, the organic solvent is methanol and the concentration of methanol is 0.1-10 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 1-9 mM, 2-8 mM, 3-7 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM; or 0.25-5 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, or about 5 mM; or 1-5 mM, for example 1-3 mM, 2-4 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, or about 5 mM.

[0066] In another example, the organic solvent is 2-propanol and the concentration of 2-propanol is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM,

about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or 1-2.5 mM, for example 1-2 mM, 1.5-2.5 mM, about 1 mM, about 1.5 mM, about 2 mM, or about 2.5 mM.

- 5 [0067] In another example, the organic solvent is acetone and the concentration of acetone is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or 1-2.5 mM, for example 1-2 mM, 1.5-2.5 mM, about 1 mM, about 1.5 mM, about 2 mM, or about 2.5 mM.
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- 20 [0068] In another example, the organic solvent is MDP and the concentration of MDP is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM; or about 4 mM.
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[0069] In another example, the organic solvent is EDTA and the concentration of EDTA is 0.1-5 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 0.5-4 mM, 1-3 mM, about 0.1 mM, about 0.5 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM; or 0.25-1 mM, for example 0.25-0.5 mM, 0.5-0.75 mM, 0.75-1 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, or about 1 mM.

[0070] In another example, the organic solvent is urea and the concentration of urea is 0.1-15 mM, for example 0.1-2 mM, 1-3 mM, 2-4 mM, 3-5 mM, 4-6 mM, 5-7 mM, 6-8 mM, 7-9 mM, 8-10 mM, 9-11 mM, 10-12 mM, 11-13 mM, 12-14 mM, 13-15 mM, 1-13 mM, 2-12 mM, 3-11 mM, 4-10 mM, 5-9 mM, about 0.1 mM, about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM; or 0.25-10 mM, for example 0.25-1 mM, 0.5-1.25 mM, 0.75-1.5 mM, 1-2 mM, 1.5-2.5 mM, 2-3 mM, 2.5-3.5 mM, 3-4 mM, 3.5-4.5 mM, 4-5 mM, 4.5-5.5 mM, 5-6 mM, 5.5-6.5 mM, 6-7 mM, 6.5-7.5 mM, 7-8 mM, 7.5-8.5 mM, 8-9 mM, 8.5-9.5 mM, 9-10 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 5.5 mM, about 6 mM, about 6.5 mM, about 7 mM, about 7.5 mM, about 8 mM, about 8.5 mM, about 9 mM, about 9.5 mM, or about 10 mM.

[0071] The protein of the buffer is casein.

[0072] In one example, the concentration of casein disclosed herein is 0.001-10 mg/mL, or 0.01-1 mg/mL. In some examples, the concentration of each of the proteins as disclosed herein is 0.001-0.005 mg/mL, 0.0015-0.003 mg/mL, 0.003-0.006 mg/mL, 0.005-0.01 mg/mL, 0.006-0.015 mg/mL, 0.01-0.03 mg/mL, 0.015-0.03 mg/mL, 0.03-0.05 mg/mL, 0.03-0.06 mg/mL, 0.04-0.06 mg/mL, 0.05-0.07 mg/mL, 0.06-0.08 mg/mL, 0.07-0.09 mg/mL, 0.08-0.1 mg/mL, 0.1-0.3 mg/mL, 0.2-0.4 mg/mL, 0.3-0.5 mg/mL, 0.4-0.6 mg/mL, 0.5-0.7 mg/mL, 0.6-0.8 mg/mL, 0.7-0.9 mg/mL, 0.8-1 mg/mL, 1-2 mg/mL, 2-3 mg/mL, 3-4 mg/mL, 4-5 mg/mL, 5-6 mg/mL, 6-7 mg/mL, 7-8 mg/mL, 8-9 mg/mL, 9-10 mg/mL, about 0.001 mg/mL, about 0.005 mg/mL, about 0.01 mg/mL, about 0.02 mg/mL, about 0.03 mg/mL, about 0.04 mg/mL, about 0.05 mg/mL, about 0.06 mg/mL, about 0.07 mg/mL, about 0.08 mg/mL, about 0.09 mg/mL, about 0.1 mg/mL, about 0.2 mg/mL, about 0.3 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, about 0.6 mg/mL, about 0.7 mg/mL, about 0.8 mg/mL, about 0.9 mg/mL, about 1 mg/mL, about 2 mg/mL, about

2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 6.5 mg/mL, about 7 mg/mL, about 7.5 mg/mL, about 8 mg/mL, about 8.5 mg/mL, about 9 mg/mL, about 9.5 mg/mL, or about 10 mg/mL.

5 [0073] In another example, the concentration of casein is 0.005-0.05 mg/mL, or 0.0015-0.06 mg/mL, for example 0.0015-0.003 mg/mL, 0.003-0.006 mg/mL, 0.005-0.01 mg/mL, 0.006-0.015 mg/mL, 0.015-0.03 mg/mL, 0.03-0.06 mg/mL, 0.04-0.05 mg/mL, 0.01-0.03 mg/mL, 0.02-0.04 mg/mL, 0.03-0.05 mg/mL, about 0.001 mg/mL, about 0.0015 mg/mL, about 0.005 mg/mL, about 0.1 mg/mL, about 0.02 mg/mL, about 0.03
10 mg/mL, about 0.04 mg/mL, about 0.05 mg/mL, or about 0.06 mg/mL.

[0074] The buffer as disclosed herein has a PH of between 6.0 and 8.0. In one example, the buffer as disclosed herein has a PH of about 6.0. In another example, the buffer as disclosed herein has a PH of about 6.5. In another example, the buffer as disclosed herein has a PH of about 7.0. In another example, the buffer as disclosed herein has a PH of about 7.5. In another example, the buffer as disclosed herein has a PH of about 8.0.
15

[0075] The buffer as disclosed herein possess unique advantages of enhancing the enzymatic cleavage activity of a Cas protein as disclosed herein by 1.5-20 fold, for example 1.76-3.04 fold, 1.88-2.70 fold, or 10.4-11.2 fold; for example 1.5-2 fold, 1.76-2.5 fold, 2-4 fold, 3-5 fold, 4-6 fold, 5-7 fold, 7-9 fold, 8-10 fold, 9-11 fold, about 1.2
20 fold, about 1.5 fold, about 1.76 fold, about 1.88 fold, about 1.9 fold, about 2.03 fold, about 2.21 fold, about 2.24 fold, about 2.6 fold, about 2.67 fold, about 2.70 fold, about 3 fold, about 3.04 fold, about 3.9 fold, about 4 fold, about 5 fold, about 5.5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 10.4 fold, about 11 fold, about 11.2 fold, about 12 fold, about 13 fold, about 14 fold, about 15 fold, about 16 fold,
25 about 17 fold, about 18 fold, about 19 fold, about 20 fold, compared to the enzymatic cleavage activity of a Cas protein in the absence of the buffer as disclosed herein.

[0076] In one example, the buffer comprises the base composition as disclosed herein, and one or more salts as disclosed herein. In another example, the buffer comprises the base composition as disclosed herein, and one salt as disclosed herein. In another
30 example, the buffer comprises the base composition as disclosed herein, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 salts as disclosed herein.

[0077] In another example, the buffer comprises the base composition as disclosed herein, and one or more organic solvents disclosed herein. In another example, the buffer comprises the base composition as disclosed herein, and one organic solvent as disclosed herein. In another example, the buffer comprises the base composition as disclosed herein, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 organic solvents as disclosed herein.

[0078] In another example, the buffer comprises the base composition as disclosed herein, and the protein disclosed herein which is casein.

[0079] In another example, the buffer comprises the base composition as disclosed herein, one or more salts (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 salts) as disclosed herein, and one or more organic solvents (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 organic solvents) as disclosed herein.

[0080] In another example, the buffer comprises the base composition as disclosed herein, one or more salts (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 salts) as disclosed herein, and the protein which is casein as disclosed herein.

[0081] In another example, the buffer comprises the base composition as disclosed herein, one or more organic solvents (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 organic solvents) as disclosed herein, and the protein which is casein as disclosed herein.

[0082] In another example, the buffer comprises the base composition as disclosed herein, one or more salts (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 salts) as disclosed herein, one or more organic solvents (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 organic solvents) as disclosed herein, and the protein such as casein as disclosed herein.

[0083] In another aspect, the present disclosure refers to a detection kit for a nucleic acid, comprising:

(a) the buffer as disclosed herein;

(b) a Cas protein selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c, Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f; and

(c) a crRNA configured to target the nucleic acid and form a complex with the Cas protein.

[0084] As used herein, "kit" means a collection of at least one component constituting the kit. Together, the components constitute a functional unit for a given purpose.

Individual member components may be physically packaged together or separately. For example, a kit comprising an instruction for using the kit may or may not physically include the instruction with other individual member components. Instead, the instruction can be supplied as a separate member component, either in a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation.

[0085] The detection kit comprises the buffer as disclosed herein which can enhance the enzymatic cleavage activity of a Cas protein selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c, Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f, a Cas protein disclosed herein, and a crRNA configured to target the nucleic acid and form a complex with the Cas protein.

[0086] As used herein, the term "CRISPR RNA (crRNA)" refers to a crucial component of the CRISPR-Cas system. crRNA is transcribed from the CRISPR locus, which contains a series of short, repetitive DNA sequences (direct repeat) interspaced with unique sequences called protospacers or spacers. These spacers are derived from previous infections by viruses or other mobile genetic elements. crRNA consists of a repeat-derived sequence (direct repeat) and a spacer sequence (protospacer). The repeat-derived sequence is conserved, while the spacer sequence is complementary to the target DNA sequence. The degree of complementarity between a crRNA and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting examples of which include the Smith- Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows- Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). The primary role of crRNA is to guide the Cas nuclease (such as Cas13 or Cas12) to the target nucleic acid such as DNA or RNA during the interference step of the CRISPR-Cas immune response. The crRNA forms a complex with the Cas protein (such as Cas13 or Cas12), and this complex then binds to the target nucleic acid DNA or RNA sequence, leading to its cleavage and subsequent degradation.

[0087] The crRNA is configured to target the nucleic acid and form a complex with the Cas protein. As used herein, the term "target" means "binding", which can refer to non-covalent physical association of a first and a second moiety (such as a crRNA and a target sequence, or a CRISPR-Cas complex and a target sequence) wherein the association between the first and second moieties is at least 2 times as strong, at least 5 times as strong as, at least 10 times as strong as, at least 50 times as strong as, at least 100 times as strong as, or stronger than the association of either moiety with most or all other moieties present in the environment in which binding occurs. Binding of two or more entities may be considered specific if the equilibrium dissociation constant, K_d , is 10^{-3} M or less, 10^{-4} M or less, 10^{-5} M or less, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, or 10^{-12} M or less under the conditions employed, e.g., under physiological conditions such as those inside a cell or consistent with cell survival. In some embodiments, specific binding can be accomplished by a plurality of weaker interactions (e.g., a plurality of individual interactions, wherein each individual interaction is characterized by a K_d of greater than 10^{-3} M).

[0088] The term "complex" refers to the complex formed by the combination of CRISPR RNA (crRNA) and the Cas12 or Cas13 protein as disclosed herein, i.e. a ribonucleoprotein complex. The crRNA can hybridize with the target sequence and thus the complex recognizes and cleaves polynucleotides that hybridize to the crRNA.

[0089] In one example, the Cas protein and the crRNA are lyophilized for easy transportation and storage, and can be reconstituted by water or a suitable buffer upon usage.

[0090] Thus, in the context of the formation of a CRISPR-Cas12 or CRISPR-Cas13 complex, "target sequence" refers to a polynucleotide such as DNA or RNA targeted by a crRNA, e.g., a polynucleotide sequence complementary to the crRNA, wherein the hybridization between the target sequence and the crRNA will promote Cas protein to exert its activity, such as the activity of cutting the target sequence. Full complementarity is not required, as long as there is sufficient complementarity to cause hybridization and facilitate Cas protein to exert its activity. A target sequence can include any polynucleotide, such as DNA or RNA. In some examples, the target sequence is located in the nucleus or cytoplasm of a cell. In some examples, the target sequence may be located in an organelle of a eukaryotic cell such as the mitochondria or chloroplast. In

some examples, the target sequence can be a sequence encoding a gene product (e.g. protein) or a non-coding sequence (e.g. regulatory polynucleotide or dummy DNA). In one example, the nucleic acid to be detected by the detection kit as disclosed herein is DNA. In another example, the nucleic acid to be detected by the detection kit as disclosed
5 herein is RNA.

[0091] In another example, the detection kit may further comprise a detecting agent comprising a probe nucleic acid, wherein the detecting agent generates a detectable signal when the probe nucleic acid is cleaved by the Cas protein such as a Cas12 or Cas13 protein as disclosed herein. In one example, the probe nucleic acid is a fluorescent probe, wherein
10 the 5' end of the fluorescent probe sequence is labeled with a fluorescent group, and the 3' end of the fluorescent probe sequence is labeled with a quenching group. In some examples, the fluorescent group is selected from the group consisting of FAM, VIC, HEX, TRT, Cy3, Cy5, ROX, JOE and Texas Red, and the quenching group is selected from the group consisting of IABkFQ, DABCYL, MGB, BHQ-1, BHQ-2 and BHQ-3. The effect
15 of the fluorescent probe in the CRISPR detection system is that after the probe is nonspecifically cut by Cas protein, the fluorescent group and the quenching group are separated, the quenching group removes the blocking effect on the fluorescent group, and the change of fluorescence intensity can be detected by means of a fluorescent signal detection device under the condition of light irradiation with specific wavelength. In
20 another example, /56-FAM/rUrUrUrUrU/3IABkFQ/ is used as a fluorescent probe to detect the enzymatic cleavage activity of Cas13. A fluorescence signal generated upon cutting of a fluorescent probe can be detected by means of a fluorescence detection equipment such as a fluorescence spectrometer, fluorometer, fluorescence microscopes, microplate reader, real-time PCR, flow cytometry, and fluorescence scanners.

[0092] In another example, the probe nucleic acid is a colorimetric probe. In some examples, the colorimetric probe comprises FAM and Biotin. In another example, the colorimetric probe can be detected by a lateral flow strip. Lateral flow strips use gold nanoparticles or colored latex beads conjugated with detection molecules. Cleavage events by CRISPR-Cas lead to a visible color change on the strip. In another example,
30 /56-FAM/CCCCCCCC/3Biotin/ is used as a colorimetric probe to detect the enzymatic cleavage activity of Cas12a. In another example, the colorimetric probe used to detect the enzymatic cleavage activity of Cas13 is selected from the group consisting of /56-

FAM/rUrUrUrUrU /3Bio/, /56-FAM/rUrUrUrUrU /3DIG/, /56-FAM/rArArArArA /3Bio/, and /56-FAM/rArArArArA /3DIG/. A colorimetric signal can be directly visually detected by naked eyes without an instrument, or with a colorimetric reader such as a colorimeter, spectrophotometer, color analyzer, chromameter, etc.

5 [0093] In another example, the probe nucleic acid has a concentration of 100-3200 nM, for example about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 800 nM, about 1000 nM, about 1200 nM, about 1600 nM, about 2000 nM, about 2400 nM, about 2800 nM, about 3000 nM, or about 3200 nM.

[0094] In another example, CRISPR-Cas cleavage events can be transduced into an
10 electrochemical signal using modified electrodes or nanoparticle-based sensors.

[0095] The shelf-life of the Cas protein and the crRNA in the detection kit is increased by 30-300% in the presence of the buffer as disclosed herein, compared to the self-life of the Cas protein and the crRNA in the absence of the buffer as disclosed herein. In some examples, in the presence of the buffer as disclosed herein, the shelf-life of the Cas protein
15 and the crRNA in the detection kit is increased by 30-100%, 50-150%, 100-200%, 150-250%, 200-300%, compared to the self-life of the Cas protein and the crRNA in the absence of the buffer as disclosed herein.

[0096] In another example, the detection kit further comprises nucleic acid amplification primers, wherein the nucleic acid amplification primers are PCR, RPA or
20 LAMP primer pairs.

[0097] The disclosed CRISPR-Cas based detection system is compatible with lateral flow strips for visual detection of the detectable signal, making it highly suitable for point-of-care testing applications. This combination of high sensitivity, specificity, and ease of use makes the method particularly valuable for detecting low-abundance nucleic acids in
25 diagnostics and other applications.

[0098] In another example, the Cas protein, such as Cas12 or Cas13 protein and the crRNA are in the form of a ribonucleoprotein complex. In another example, the concentration of the Cas protein used to form ribonucleoprotein complex is 200-3200 nM, for example, about 200 nM, about 250 nM, about 300 nM, about 350 nM, about 400 nM,
30 about 450 nM, about 500 nM, about 550 nM, about 600 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, about 950 nM, about 1000 nM, about 1050 nM, about 1100 nM, about 1150 nM, about 1200 nM, about 1250

nM, about 1300 nM, about 1350 nM, about 1400 nM, about 1450 nM, about 1500 nM, about 1550 nM, about 1600 nM, about 1650 nM, about 1700 nM, about 1750 nM, about 1800 nM, about 1850 nM, about 1900 nM, about 1950 nM, about 2000 nM, about 2050 nM, about 2100 nM, about 2150 nM, about 2200 nM, about 2250 nM, about 2300 nM, about 2350 nM, about 2400 nM, about 2450 nM, about 2500 nM, about 2550 nM, about 2600 nM, about 2650 nM, about 2700 nM, about 2750 nM, about 2800 nM, about 2850 nM, about 2900 nM, about 2950 nM, about 3000 nM, about 3050 nM, about 3100 nM, about 3150 nM, or about 3200 nM. In another example, the concentration of crRNA used to form ribonucleoprotein complex is 200-3200 nM, for example, about 200 nM, about 250 nM, about 300 nM, about 350 nM, about 400 nM, about 450 nM, about 500 nM, about 550 nM, about 600 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, about 950 nM, about 1000 nM, about 1050 nM, about 1100 nM, about 1150 nM, about 1200 nM, about 1250 nM, about 1300 nM, about 1350 nM, about 1400 nM, about 1450 nM, about 1500 nM, about 1550 nM, about 1600 nM, about 1650 nM, about 1700 nM, about 1750 nM, about 1800 nM, about 1850 nM, about 1900 nM, about 1950 nM, about 2000 nM, about 2050 nM, about 2100 nM, about 2150 nM, about 2200 nM, about 2250 nM, about 2300 nM, about 2350 nM, about 2400 nM, about 2450 nM, about 2500 nM, about 2550 nM, about 2600 nM, about 2650 nM, about 2700 nM, about 2750 nM, about 2800 nM, about 2850 nM, about 2900 nM, about 2950 nM, about 3000 nM, about 3050 nM, about 3100 nM, about 3150 nM, or about 3200 nM.

[0099] In another aspect, the present disclosure refers to a method of detecting a nucleic acid in a sample by clustered regularly interspaced short palindromic repeats and CRISPR associated protein (CRISPR-cas), using the detection kit as disclosed herein, the method comprising: incubating the sample containing the nucleic acid with the Cas protein and the crRNA, wherein when the nucleic acid is present in the sample, enzymatic cleavage activity of the Cas protein is induced to generate a detectable signal when the probe nucleic acid is cleaved; wherein the buffer enhances the enzymatic cleavage activity of the Cas protein.

[00100] As used herein, enzymatic cleavage activity of a Cas protein refers to its ability to indiscriminately cleave single-stranded nucleic acids in the vicinity once it has been activated by recognizing and binding to a specific target nucleic acid sequence. This activity is also known as trans-cleavage activity.

[00101] In one example, the nucleic acid in the sample can be amplified using a technology selected from the group consisting of Polymerase Chain Reaction (PCR), Recombinase Polymerase Amplification (RPA), Loop-Mediated Isothermal Amplification (LAMP), and Rolling Circle Amplification (RCA) prior to carrying out the detection method as disclosed herein. In another example, the method of detecting a nucleic acid in a sample as disclosed herein does not comprise pre-amplifying the nucleic acid in the sample.

[00102] In another example, the method of detecting a nucleic acid in a sample further comprising using a detection method selected from the group consisting of a colorimetric detection method, a fluorescent detection method and an electrochemical detection method to detect the detectable signal which is a colorimetric signal, a fluorescent signal, or an electrochemical signal. In another example, the colorimetric detection method uses a lateral flow strip and a colorimetric probe comprising FAM and Biotin. In another example, the fluorescent detection method uses a fluorescent probe, wherein the fluorescence signal is measured using a fluorometer, a microplate reader, or Real-Time PCR. In another example, the electrochemical detection method transduces CRISPR-Cas cleavage event to an electrochemical signal using a modified electrode or a nanoparticle-based sensor.

[00103] In one example, the Cas protein used in the detection kit to be used in the method of detecting a nucleic acid in a sample as disclosed herein, is a Cas12 protein selected from the group consisting of Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f. In another example, the Cas protein used in the detection kit to be used in the method of detecting a nucleic acid in a sample as disclosed herein, is a Cas13 protein selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c. In another example, the Cas13d protein is selected from the group consisting of Cas13-Cd13 having an amino acid sequence of SEQ ID NO: 2, Cas13-Cd14 having an amino acid sequence of SEQ ID NO: 4, and Cas13-Cd15 having an amino acid sequence of SEQ ID NO: 6.

[00104] In one example, the method of detecting a nucleic acid in a sample as disclosed herein is performed at a temperature of 20-60 °C. In another example, the method of detecting a nucleic acid in a sample as disclosed herein is performed at a temperature selected from the group consisting of 20-25 °C, 25-30 °C, 30-35 °C, 35-40 °C, 40-45 °C, 45-50 °C, 50-55 °C, 55-60 °C, about 20 °C, about 25 °C, about 30 °C, about 35 °C, about

37 °C, about 40 °C, about 45 °C, about 50 °C, about 55 °C, and about 60 °C. In one particular example, the method of detecting a nucleic acid in a sample as disclosed herein is performed at a temperature of about 37 °C.

[00105] In one example, the nucleic acid to be detected by the detection method as disclosed herein is DNA and the DNA is 10 copies/ μ L or less, for example, as low as about 0.0015 copies/ μ L. In another example, the DNA to be detected method as disclosed herein is 0.0015-0.01 copies/ μ L, 0.0075-0.015 copies/ μ L, 0.01-0.015 copies/ μ L, 0.015-0.15 copies/ μ L, 0.15-1.5 copies/ μ L, 1-3 copies/ μ L, 1.5-3.5 copies/ μ L, 2-4 copies/ μ L, 2.5-4.5 copies/ μ L, 3-5 copies/ μ L, 3.5-5.5 copies/ μ L, 4-6 copies/ μ L, 4.5-6.5 copies/ μ L, 5-7 copies/ μ L, 5.5-7.5 copies/ μ L, 6-8 copies/ μ L, 6.5-8.5 copies/ μ L, 7-9 copies/ μ L, 7.5-9.5 copies/ μ L, 8-10 copies/ μ L, less than 0.0015 copies/ μ L, less than 0.0075 copies/ μ L, less than 0.01 copies/ μ L, less than 0.015 copies/ μ L, less than 0.1 copies/ μ L, less than 0.15 copies/ μ L, less than 1 copies/ μ L, less than 1.5 copies/ μ L, less than 2 copies/ μ L, less than 3 copies/ μ L, less than 5 copies/ μ L, less than 6 copies/ μ L, less than 10 copies/ μ L, less than 15 copies/ μ L, less than 20 copies/ μ L, less than 30 copies/ μ L, less than 40 copies/ μ L, less than 50 copies/ μ L, less than 60 copies/ μ L, less than 70 copies/ μ L, less than 80 copies/ μ L, less than 90 copies/ μ L, less than 100 copies/ μ L, or less than 150 copies/ μ L. In another example, the DNA to be detected by the detected method as disclosed herein is about 0.0015 copies/ μ L, about 0.0075 copies/ μ L, about 0.01 copies/ μ L, about 0.015 copies/ μ L, about 0.1 copies/ μ L, about 0.15 copies/ μ L, about 0.2 copies/ μ L, about 0.3 copies/ μ L, about 0.4 copies/ μ L, about 0.5 copies/ μ L, about 0.6 copies/ μ L, about 0.7 copies/ μ L, about 0.8 copies/ μ L, about 0.9 copies/ μ L, about 1 copies/ μ L, about 1.5 copies/ μ L, about 2 copies/ μ L, about 2.5 copies/ μ L, about 3 copies/ μ L, about 3.5 copies/ μ L, about 4 copies/ μ L, about 4.5 copies/ μ L, about 5 copies/ μ L, about 5.5 copies/ μ L, about 6 copies/ μ L, about 6.5 copies/ μ L, about 7 copies/ μ L, about 7.5 copies/ μ L, about 8 copies/ μ L, about 8.5 copies/ μ L, about 9 copies/ μ L, about 9.5 copies/ μ L, about 10 copies/ μ L, about 11 copies/ μ L, about 12 copies/ μ L, about 13 copies/ μ L, about 14 copies/ μ L, about 15 copies/ μ L, about 16 copies/ μ L, about 17 copies/ μ L, about 18 copies/ μ L, about 19 copies/ μ L, about 20 copies/ μ L, about 30 copies/ μ L, about 40 copies/ μ L, about 50 copies/ μ L, about 60 copies/ μ L, about 70 copies/ μ L, about 80 copies/ μ L, about 90 copies/ μ L, about 100 copies/ μ L, about 110 copies/ μ L, about 120 copies/ μ L, about 130 copies/ μ L, about 140 copies/ μ L, about 150

copies/ μ L, or more. In another example, no DNA amplification is required before detection using the disclosed detection method.

[00106] Advantageously, the present disclosure showcases superior detection of low concentrations of DNA using the present amplification-free CRISPR-Cas-based detection system with the aid of the buffer as disclosed herein, which significantly enhanced the enzymatic cleavage activity of the Cas protein such as Cas12 or Cas13.

[00107] In another example, the sample comprising the nucleic acid is a biological sample obtained from a tissue sample, saliva, blood, plasma, milk, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, nasal swab specimen, or swab of skin or a mucosal membrane surface or a combination thereof. In another example, the biological sample is a crude sample. In another example, the nucleic acid is not purified or amplified from the biological sample.

[00108] In another example, the sample comprising the nucleic acid is a chemical sample is obtained from a pharmaceutical substance, a chemical reagent, a cosmetic product, an industrial effluent, a pollutant, a hazardous material, a polymer, an adhesive, a solvent, an oil, a gas, a metal alloy, a nanomaterial, a fuel, a cleaning agent, a dye, or a combination thereof. In another example, the chemical sample is a crude sample. In another example, the nucleic acid is not purified or amplified from the chemical sample.

[00109] In another example, the sample comprising the nucleic acid is an environmental sample is obtained from a food, a beverage, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a freshwater sample, a waste water sample, a saline water sample, exposure to atmospheric air or other gas sample, or a combination thereof. In another example, the environmental sample is a crude sample. In another example, the nucleic acid is not purified or amplified from the environmental sample.

[00110] In another example, the nucleic acid in the sample belongs to a microbe selected from a bacterium, a virus, an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion. In another example, the nucleic acid in the sample belongs to a plant, an animal, an insect, a microbe as disclosed herein, or a combination thereof.

[00111] In another example, the virus is selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus.

- [00112] In another example, the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human
- 5 respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mmarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus,
- 10 Avian paramyxoviruses, penguin or Falkland Islandsvirus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Betacoronavirus, Betapapillomavirus 1-6, Bhanja virus, Bokeloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepacivirus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus,
- 15 Caliciviridae virus, California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chaparc mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade
- 20 virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Fer-de- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarenavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarenavirus, Hantaan virus,
- 25 Hantavirus Z10, Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus, Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human picomavirus, Human smacovirus, Ikoma lyssavirus, Ilheus
- 30 virus, Influenza A-C, Ippy mammarenavirus, Irkut virus, J-virus, JC polyomavirus, Japanese encephalitis virus, Junin mammarenavirus, KI polyomavirus, Kadipiro virus, Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur forest

disease virus, Lagos bat virus, Langat virus, Lassa mammarenavirus, Latino mammarenavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu virus, Louping ill virus, Lujo mammarenavirus, Luna mammarenavirus, Lunk virus, Lymphocytic choriomeningitis mammarenavirus, Lyssavirus Ozemoe, MSS12Y225

5 virus, Machupo mammarenavirus, Mamastrovirus 1, Manzanilla virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory syndrome coronavirus, Mobala mammarenavirus, Modoc virus, Moijang virus, Mokolo virus, Monkeypox virus, Montana myotis leukoencephalitis virus, Mopeia lassa virus reassortant 29, Mopeia

10 mammarenavirus, Morogoro virus, Mossman virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus, Nariva virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat hepacivirus, Ntaya virus, O'nyong-nyong virus, Oliveros mammarenavirus, Omsk hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana mammarenavirus, Parramatta River virus, Peste-des-petits-

15 ruminants virus, Pichande mammarenavirus, Picomaviridae virus, Pirital mammarenavirus, Piscihepevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta Toro virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile bomavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo virus,

20 Rodent Torque Teno virus, Rodent hepacivirus, Ross River virus, Rotavirus A-I, Royal Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus, Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik virus, Severe acute respiratory syndrome-related coronavirus, Severe fever with thrombocytopenia syndrome virus,

25 Shamonda virus, Shimoni bat virus, Shuni virus, Simbu virus, Simian torque teno virus, Simian virus 40-41, Sin Nombre virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taila virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-

30 borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus,

- Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus, Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or *Zygosaccharomyces bailii* virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus, Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof. In a particular example, the coronavirus is SARS-CoV-2. In another particular example, the nucleic acid in the sample is a SARS-CoV-2 N gene or a fragment thereof. In another example, the nucleic acid in the sample is a SARS-CoV-2 N gene or a fragment thereof and comprise a sequence of SEQ ID NO: 14.
- [00113] In another example, the bacteria is a Gram-positive bacterium or a Gram-negative bacterium selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile* and *Clostridium tetani*, *Corynebacterium diphtheria*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Chlamydia trachomatis*, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*, and *Vibrio cholera*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Mycoplasma mycoides*, *Clostridium perfringens*, *Mycobacterium avium* *Salmonella* spp., *Pasteurella multocida*, *Haemophilus parasuis*, *Actinobacillus*

pleuropneumoniae, *Yersinia pestis*, *Francisella tularensis*, *Bacillus anthracis*, *Coxiella burnetii*, *Listeria monocytogenes*, *Clostridium chauvoei*, *Campylobacter* spp., *Chlamydia psittaci*, *Anaplasma marginale*, *Leptospira* spp. Causes leptospirosis, *Bordetella bronchiseptica*, *Clostridium perfringens*, *Staphylococcus intermedius*,
 5 *Bartonella henselae*, *Chlamydophila felis*, *Mycoplasma* spp, *Pasteurella multocida*, *Erwinia amylovora*, *Xanthomonas* spp., *Xanthomonas axonopodis*, *Ralstonia solanacearum*, *Pectobacterium* and *Dickeya* spp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas oryzae*, *Clavibacter michiganensis*, *Xylella fastidiosa*, *Pantoea* spp., *Burkholderia glumae*, *Xanthomonas citri*, *Xanthomonas translucens*, *Xanthomonas fragariae*, *Xanthomonas hortorum*, *Clavibacter michiganensis*, *Pseudomonas syringae* or a combination thereof.

[00114] In another example, the nucleic acid is a RNA or a DNA from a virus as disclosed herein, or a RNA or a DNA from a bacteria as disclosed herein; the RNA is
 15 reverse-transcribed to complementary DNA (cDNA), and then isothermally amplified using methods such as Recombinase Polymerase Amplification (RPA) or Loop-Mediated Isothermal Amplification (LAMP), before subjected to the method as disclosed herein. In another example, the nucleic acid is a cDNA. In another example, the target DNA is selected from the group consisting of single-stranded DNA, double-stranded DNA, or a
 20 combination thereof. In another example, the target DNA is a synthetic or naturally occurring DNA. In another example, the target DNA comprises a wild-type or mutant DNA. In another example, the target DNA includes DNA obtained by reverse transcription or amplification of RNA, such as cDNA.

[00115] **Industrial Applicability**

25 [00116] It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

[00117] As used in this application, the singular form “a,” “an,” and “the” include plural
 30 references unless the context clearly dictates otherwise. For example, the term “a primer” includes a plurality of primers, including mixtures and combinations thereof.

[00118] As used herein, the term “comprising” means “including.” Variations of the word “comprising”, such as “comprise” and “comprises,” have correspondingly varied meanings. Thus, for example, a composition “comprising” X may consist exclusively of X or may include one or more additional unrecited components.

5 [00119] As used herein, the term “about” in the context of concentration of a substance, size of a substance, length of time, or other stated values means +/- 5% of the stated value, or +/- 4% of the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value.

[00120] Throughout this disclosure, certain embodiments may be disclosed in a range
10 format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to
15 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[00121] The invention illustratively described herein may suitably be practiced in the
20 absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including”, “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of
25 the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied herein disclosed may be resorted to by those skilled in the art, and that such
30 modifications and variations are considered to be within the scope of this invention.

[00122] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form

part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00123] Unless otherwise indicated, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

[00124] Other embodiments are within the following claims and non-limiting examples.

EXAMPLES

[00125] Non-limiting examples of the disclosure will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the disclosure.

Example 1: Expression and Purification of Cas13d (Cd14) enzyme

[00127] The Cas13 enzymes (Cd13, Cd14, and Cd15) (disclosed in PCT/SG2022/050859, sequence can be seen in Table 1) were engineered with a dual affinity tag system: an N-terminal His6-MBP (hexa-histidine tag for purification and maltose binding protein for solubility enhancement) with a Tobacco Etch Virus (TEV) protease cleavage site, and a C-terminal enterokinase (EK) cleavage site followed by another His6 tag. These constructs were synthesized by GenScript (USA) and cloned into the pET21a(+) vector between the NdeI and NotI restriction sites. The resulting plasmids, pET21a(+)-His6-MBP-TEV-Cas13-EK-His6, was then introduced into Escherichia coli BL21 (DE3) cells for protein production.

Table 1. The list of sequences for Cas13 enzymes.

SEQ ID NO	Description	Sequence
1	Cd13 DNA sequence	GTGAGCAAGAATGATAACATCAAATCCAAAGCAAAGCACTTGGCTTGAAGTCAACGTTTCA GGTTGGCGACGAGGTCGTTATGACTTCTTTCCGGTAAAGGTAATAAGCCATCGTTGAAAAG ATTGTACGTGGAACCGACGTGCAGTCTGTTCCGACTGAGCCGAACCTCAGCGCTGAAATCG AAGGTAAAAATTTGACCTTGTCCGCGACAGCTCATATTGACACAAAGTCGGACAATCCTCAG TATTCAAAGAAAAGAACCGGCCGACGATATGATAGGCGCAAAGGCGGCACTTGAAAAGAGAT TCTTCGGCGGTACGTTTGACGACAATATACATATTGAGCTCGCTTACAATGTTCTTGATATT GAAAAGATTCTTCTGTCCATATCAACAATATTGTTTACACGCTTAATAACATACGAAGAAAA GACAACGCTGAAGATGACGATTTTATCGGCTATATGAGTACCAGAAACGACTACGACACCT TTATTGAACCGAGAAAGCACACATTAGCGAAGACGCTGCTAAAAGCATTGACAAATCAAGA GCCTCGTTTGAAGAGTATCTTCAGCCGCCGGTAAAGGATTGTCTGCATTATTTGCGAAACA CCTTTTGTCTCCCGTGAGATTGAAAAGACATACACGATAATCGCGGCTTTGAAAGGAA GAAAAAAGTTACCGTAAACTCGCTTATAGACGAGAAGGAATATACTATATTTTGGCCTCC TCGGCGGACTTCGTCAATTCTGTACCCATGACAACAAAAGCGGCAGAACTGGCTTTATTC TCTCGAGGAAACGGAATAAACTCTGATGCAAGGCGGTACTTGACAAGTACTATAATTCTG CAGTTGCAAGGATTGACGAAAGCTTTGTTGACAACGCATCTAAAACATAATTTCAAGCTGATT

		TTTAATGCAATGGATGTCAGCGACGAAGCGCTTCAGGACAATATAGCAAAGGCGTTTACAG AGTTTACGGTTTGTAAAGAGCTTTAAAAACATGGGCTTTTCAATTAAAAAGCTCCGTGAACAG CTGCTCGAAGCTTCTGGAATATGAAGGGCTTAAAGATAAGCATTACGATTCGGTGGCTCAAA GCTTTATCAGATTATGGACTTCGTTATTTATCTGAGTTTCAAAGATGAAAAGTTTAAAGAGGA TAATGAAGAAAAGATTAAATAATATCGTTAACGAACTCCGTGCGACCTTGAACGAAAGGATA AAGGCAGAGTATATGCTCTTATGCCGAAAGGATGAAATCTGAGCTTAAACCGGCAATTGC ACGCCCTTAAGTCTGATATTGACAAAATTAAGGACAGCAGGGTGAAAGAGTTTGAACCTTGATG CGTCCGTAAAATATAGACTCTCAAAGGTCGTAGAAAGTGCCGCTTAAGGATAGGGCAAC ATATTTTCAAAGCTCATATATCTGACTACTCTGTTCCCTGACGGCAAGGAGATTAAAGACC TCCTGACTACTCTTATTATCATCAGTTTGAGAATATCGCAAGTTTATTTGATGTTTGAACGACA GAGGTATTGATTGCCGTTTTTCGGATGGATATAAGCTTTTGAATCCCTCAAAGCAAAATGCG TTTGAGCTTCGCAATGTCAATAGCTTTGCTCGTATGACAAGGACCTCAAAGGATGACGAAAA CGCAACGCATATGATGATATAGATGCTGCAGAGATTCTCGGCACCGATTATACCGAAGAG CAGATTGAGGAGCATCTTAACCTGGAAAAGAAAAGAAATGATACCGACGACGAAAAGCGG ATATGAACCTCCGTAACCTTTATAATCAATAACGTTATAAAGTCATCAGCTTCAATTATCTTGT CAGATATTTCAATCCTAAAAAAATCAGAGCGCTCGCCGATAACGAGGGTGTATCCGTTTTG TCCTGGGGCAGTTGCCGATGCGCAGATTGACCGTTATACTTTACTTTGCCGGCTTCAATCG CGATGCCGACCGGACGAGAAAAACGGACAAACTTGCAAAGGCAATTAAGTTTGAAGTTT AACGATTTTGAATAATGTTAAACAGGGCGCAAACTAGGGGCGCAATCTCAGGAGTCAATTG ACAAGGCACAGAAGCAGGGACTGATTTCTTGTACTTAACCGTTCTATATCTTTTGACAAAG AATCTTGTATTGTAACCTACCGCTATTTCTTGCATTCCATTGCTTTCAGCGGTGATGCGCAG CTGCTTGGAGCGGTGCTGGGCATCATGAGCCTTATGTTGCTCTTACTCAGCGCTTATTAA TGAAGATAAGGCTCAATGAGCATGCTGCGAATATCTTAAGACCAATATTGCAAAATTCGGATG AATATACAATTAGAATTCCGCAATAATGCTGCTCACTTGAGTGCTGTAGAAATGCAAAATC TGATATTGACAAGTTGAAGGAATTTAAATCGTACTATGAGATTATCATTTCCTTTCTCAAG AGAATATTTACCGTAAATATTGCGTTGATAAGAAATATGTTACAGCCGATGAAATGGAAGT AAAACAATAAGTGTAAAAATTAGCAAGGATTATTGCTCCTCAGGTATATATCGACAAGTCGCT GAATACTTTGACAAGCTCAATAAATATGGTACGATTGCAAGGACTTCACGAAAGGCACTCAA TTCGCCATTGCGCTACAACCTCGCAAGATATAAGAAATCTGTCTATCGAGGGCTTGTTCGACC GAAACCGTCCCGAGACAAAGGTGAGAACACGTTTGAGGACTAA
2	Cd13 amino acid sequence	VSKNDNIKSKAKALGLKSTFQVGVDEVMTSFGKGNKAIVEKIVRGTDVQSVPTPEPNFSAIEGKK FDLVGRAHIQTKSDNPQYSKRTGDDMIGAKALEKRFFGGTFDDNIHQLAYNVLDIEKILSVHI NNIVYTLNNIRRKDNAEDDDFIGYMSTRNDYDTFIEPRKHNISEDAKSIDKSRASFEELYLQPPVK DCLHYFGNTFFAPRIEIKTYTDNRGFERKKKVTNLSLIDEKEIYYIFALLGLRQFCTHDNKSGR NWLYSLEENGINSDAKAVLDKYYNSAVARIDESFVDNASKTNFKLIFNAMDVSEALQDNIKAF YKFTVCKSFKNMGFSIKKLREQLLEPEYGLKDKHYDSVRSKLYQIMDFVIYLSFKDEKFKKDN EEKINNIVNELRATLNEEDKGRVYASYAERMKSELKPAIARLKSIDIKIDSRVKEFLDASVKYR LKSXVSVRLKDRATYFTKLILYLTFLDGLKEINDLLTLIHOFENIASFIDVMNDRGIDCRFSDGY KLFESSKQIAFELRNVSFARMTRTSKDDENATHMMYIDAEEILGTDYTEEQIEEHLNLEKKRM PGTKKADMNFRNFIINNVIKSSRFNLYVRYSNPKIRALADNEGVIRFVLGELPDAQIDRYTLCCG FNPDADROEKTDLAKAITLRFNDFENVKOGANTEGESQESIDKAOKGLSLYLVLYLLTKN LVYVNSRYFLAFHCLERDAQLLGSGAGHHEPYVALTQRFINEDKLNHACEYLKTNANSDEYTI RIFRNNAAHLSAVRNANLYIDKLKEFSYIEYHFLSQENIYGKYCVDKYVYIADENGSKTISVKI SKDYCPQVYIDKSLEYFDKLNKYGTGYCKDFTKALNSFPFYNLARYKNLSIEGLFDRNRPDOKGE NTFED
3	Cd14 DNA sequence	ATGGCGAAGAAACTTAGTCCGAAAGAAATAAGGGAAGCTGCAAAGCAGAAAAGATGAAAA GCATAAAAGCTGCAGAGGCTGAAAGAGAAAAAGCTGCTGAAGAAGCGAAACTTAAAGCTGA AGCAGAAAAGAAAGAAAAAGCAGAAAAAATGAACGCGAAAAAGCACTGAAGCGCTTCAGA CTTGATGAAAAATCACGAATGGCTCTTCTTAAAGTGAAAGAAAGTCACTTGCCAAAGCCGC TGGGGTCAAATCAGCTTTTGTGTTGGAATGACATTTATCTTACTTCATTCGATCGCGGTG ACGATGCAATCGTAGAAAAAGAAATCACTGATACAGTGGTAACAAATCTGAGAAAGTGATGAA TCATTTGAGGTAAATGAAAACTATTACAGAAATGTCTGTTCCGATAAAAAGCAACGTATA TCTGATCTGTACGCTATAGCTGACAAACCGCTTTACAGAAAAGATTACGCGACTAAAGTTCA ACCGGACAAAGCTTCTTCTCAAGGATACCTTAGAAAAACTATACTTTGAAAAAATTTTGATGA TACACTTCACATCCAGATCATATACAACATTTGAGACATTGAAAAAATACTTACCGTTTAC AGCATCAATACGATCTACTGCTTAAACAATCTGTTTGAAAAAGAAAGCGGTGAAAAAGAGA CCTTATTAGTAAACTGACCTATCAGATAACATATGATGAATTTAAAGAAAGTAAAGCTCATAA TGAATTCATCGATTTTATAATCTGAACACCTTAGGGTATTACGGCAATATTTTTTCAAAGA AAAAAGAAACGTTACAAAAAGAAATCTATGATATCATAGCTCTTATAGCTACCATAAGACA ATGGTGTGTTCACTGTGAAGAAGACAAGCGCACATGGCTGTTCAACACAGAAACCGTTTTA AGTAAGGAATTTCTTGATATTTCTTGATGACGTTTACGAAAGTCTTGTGAAAAAGTAAACAGA AATTTTCTGAAAGACAACAAGGTTAATCTGCAAACTACTCGAAGACGTCCTGGAATAAAGAA CAGTGAATCAGGTGAAAACTTATCCGACAGTACTATCGTTTCATTGTTACCAAGAACAGAGA AAGTTCTCGGGTCTCGATTAAAAAGCTTCGTGAAGCAATGCTTGAAGAAACCGAATTCAAA ACAGACAAAAAATATGATACTGTTGTTCAAAGCTTTTACAGCTTATCGATTTTCTCTGTTT ACAGGATACACTACTGATGAAGCTGAAAAAGAAAAAGCACTTTTTCTTATTAATCTCTCCG GGAATCATTGACCGAGGAACTAAAGACAGAAATACAAATCAGAAAGCTGCTCGTTTATGG CTGAAATACGAAATACTATAACCAACAAAAATCAGAGAAGCTCTCAATGAAAAATCTATAAG CGAATTAATAAAGATAAAAAGCTTCGATGATAAAGTATTACCAGCATCATAACAGATGAAG TATCAGGGAAAAAGCAACTTATTTTCAAAAAAATCTATCTTCTGCTCAGTTTATCGACG GAAAAAGTAAACGATCTGACCACTTCACTTATAACAAATTTGATAATATACGAAAGCTCA TTGATACTGCCGACAGATAGGACTTGACTGTAATTTACAGAAGAATACAAATCTTTTGAA

		AACTCCGATCAGATAAGAACAGAACTTCATGTTATAAAAAATCTTACTAACATGGAGTATTAC GATACTACTGTAAAAAACAGATGTACAAAGACGCCGTTACATTCTCGGAATACAGGATGA TGTTTCTGACGCAGAACTTGAAGAGATCATCAATTCATTCTTCTTAAATGAAACGGCAA ACCACTCCCTGGAACCAAGGTAAGGATTCGGTAATTTATCATTTCCAATGTTTTAAA ATCACGTAGATTCAATTTATCTCATAAAATCTGCAATCCTAAGAAAGATAAGAAAAATTCAGG AAACAGAAAAATCATAAGTTTCGTTTTAAGCCGTATAACAGACTCACAACTGGAAAGATACT ACTATTCATGCAATCCGGAACCTTAAATCCGGTATCTATCCGGGACGTGATGATGCTGCTAAAT GATCTTTCTATTCTATCGTGACATGAAATTCGAAGATTTTAAAAATGTTGATCAGAGCGCA AATGTTTCATGACAACAACATGCTGCCAGAGAAAAATGAAGTATCAGACTATAATCAGTCT TTATCTTACAGTTTGCTATCATCTGGTTAAAAATCTTGTAACATAAACGCCAGGTATGCTAT GGCCTTCCACGCACCTTGAACGCCGACGCTCGCCTTACCAATTTTCAGCAGTGAAGAGAAT TATGTGGATAATTTAAATGCTGACTATGCTATACTTACTAAAAACACTTCTGAAAGACAACAT GAAAATGCCGGAACCTTTATCTCAGAAACAAAAATGGAATAAATCACAAGAGAGAATCT TGATAATTACATTCTCAGGCCGCTGCAAACTCAGAAACGCAGTAGCTCATCTTAAACCCGA TAAGAAATGCTGATATGCTGCTTGAAGATATTGAAGATGTATCATATATTATGCCATCTATC ATTACATCATGCGAAAAAGTGTACAAACAGAACTATAAGGGTTTCTAATACTACTGAAGAC GAAAAGCGCAATCTTACAGATTATCAGAACAAAAATCAAAAGACATCATGGTTTATAACAAAGA TTTTGTAAGAGCTCTCTGCGTGCCTTTTGCCTATAATATAGTCAGGTTCAATGCTCTTTCTAT ATATGAAATGTTTGATCGTAATTATCATGAAAAACGTCACCGGAACTGATGACAGCCAAA GCCCCTGA
4	Cd14 amino acid sequence	MAKKLSPKEIREAAKAEKMSIKAEEAEREKAAEEAKLKAEEKKEKAEKNEREKALKRFRRLDE KSRMALPKSERKSLAKAAGVKSFAVGNDIYLTSDFRGNDAIVEKKITDVTVNTLRSDSEFEVNE NTITEMSVPIKSKRISDLAIAADNPLYRKDSATKVQPDKLLKDTLEKLYFGKTFDDTLHQIYNILD IEKILTYYISINTIYCLNNLFGKESGEKEDLISKLTYYQITDEFKESKAHNEFDYFNLTLYGYGNIF KEKKRSQKEIYDIIALTIQWCVHCEEDKRTWLFNTETVLSKEFLDILDVYESLVEKVNRNF LKDKNVNLQILEDLVEIKDSERELIRQYRFIVTKEQKLGLFSIKKLREAMELETEFKDKKYDT VRSKLYKLIDFLFTGYTDEAEKEKALFLIKSLRESLTEETKDRIYKSEAARLWLKYENTITNKIRE ALNEKSISELKDKDSFDDKSITSITDEVSGKKATYFSKTIYLLAQFIDGKEVNDLTSLINKFDNIRS LIDTAGQIGLDCKFTTEYKFFENSQDQIRTELHVIKNTNMEYYDTTVKKQMYKDAVHLIGQDDVS DAELEKIINSILLNENKPLPGTKGKKGFRNFIISNVLSRRFIYLIKYNCPKIRKIAGNRKIKFVL SRITDSQLERYYYSCNPELKSIIYPRDDAVNDLSILIAMKMFEDFKNVQDSANVHNNNAARE KMKYQTIISLYLTVCYHLVKNLVNINARAYAMAFHALERDARLYQIFSEENYVDNLNADYAILTKT LLKDNENAGNLYLRNKKWNKLTRENLDNYIPQAAANFRNAVHLNPIRNDMLLEDIEDVSSY YAIYHYIMQKSVTNRTIRVSNNTTEDEKRLTDYQNKIKRRHHGYNKDFVKALCVFPAYNIVRFKSLSI YEMFDRNYHEKTSPEDDSQSP
5	Cd15 DNA sequence	ATGGATAAGGAAAAACAACTGTAGAAGGAAAAATACTAATCAAAAATCTGATGTGTTAAA ATCGCTGGCAAGAAAGCAAAATGGACTTAAATCTCTTTTGTATAGGGAATGAAGTTGTTATGA CTTCATTGGGAAGAGGAAACAGTGCGATTCTTGAGAAAAAGATAACGGGCTCGAGAAATAGA AAATCTTAATCCGAACGTTGCGTTTTATGTTAAAAACATATATCAGATAGTTGTAATCCTGA TAGTGGTAAATATGACGTAAAGAGTAAACGAATGAAGGAAAAAGCTGTTGTATGATGATCCAG TATATGTTTCTCGGAAAAAGCTTCAAAATGTTTCATGCTGGTCAGGATCTCATTTGCTGTAAAG AATGTTCTGGAAGAACGCTTATTTGGAAGAACTTTTATGATAATATCCACATATGATGCTGAT CTATAATATCTTGACATAGAAAAAGATACTTGCAGTTTCATGTTTCAAAATGCAACTTTTGCCAT TAACAATATTTTAGGAATTGAAGGCAAGGAAACGAAGATTTTATCGGAAATCTATCAGTGC TCAATACTTTTATGATGAGTTCGAGAAATATGAAACGCATCCAAAGTTTGCAACAAAAAGTGCG ATAAAAGAAAATCTCAGGAAATCCAAAGTTTTTTTGATAAGATTAAAAAGGAAATAAACTC GGATATTTTCGGTCAGGCTTTTTATTATGCAACAGGTACTGGTAAAAATCTGATATTCACCAA GAAATCTTCAGAGACGATATATGAATTAATGCTCTTGTAGGAAGTCTCAGACAAATTTTGCG TTCATGACGAGGTAAAGGTTGATAATAAAGTAAATCAAGAAGCTGGCTGTATAATGCTCAA AAAGAGTTAAAGCCAGATTTTCTAAAAGCACTTGTGAAGTGTATGTAAGAAAGTTGAAAA AATAGACAGTGATTTTATGTAATAATACAGTTGATTTGCACATTATTCATGATGCAATAGA TGTTATTGACGGATCAGCCGACTGGCAGAAAAATCACAATGAATATTATGATTTTATAATAA GAAAATGTTTTAAGAATATCGGTTTTTCTATAAAAAAGACTTCGCGAAACAAATGATTGAAGAAC AGATGAAAGTATTATGCGGAAAAATGCGATAGAGAGAACTGTAAGGTTTATGGCAAAATGCTTC AAAAACAAGAAATGATAGCGTTCCGTTCCAGATTAAACAGAAATCGTTGATTTTATAATATTC AGGCATTATAATGATGAAGCAATCTTAAAGAAATGAAGTCTTCTCAGAACCTGTATGAGTGA AGAAGAAAAACAGAAACGATTTTATTTGCGGAAGCTAAAGCTTTATGGAAAAAGTACAAAT ATGTGTTTCAGAAATATGTTCTTAAAAAATCTTAAAGGCAAGTCGATAAGCGGGTTAAAGAG AAAGCAATCGAAAAATCCATAGATATTAAGTCAAGTAAAGATAAGCCTCGGTGATCCGGATTA TTTCTGTAAGTTTATTTATCTTCTTACGTTCTTCTTGATGGTAAGGAGATAAACGATTTACTT ACAACGCTTATCAATAAGTTTGATAACATTGCAAGTTTTATAAGTGTATGAAAAATGATAAG TTATCTATTGACTGTGAATTTGTACCGGAATACAGCTTTTTCGCAAAACGAGTCTCAAAATCACA TCTGATCTCAGAGTTATAACAGTTTTGCAAGAAATGCAGGCACCGGCTGAGCCGTGCAAGAG ATGATATGTACCGGTGATGCTCTTGATATCCTTGGCATGGATGACCTGTGCAAGAGATGGAAG GAAGCAGCTTGAAGATACAGTTTTATGCAAGATGAAACGGTAAGTACATGAAAAAGAA GATAATAATCTAAACGTGATACCAACTTTAGAAATTTTCTGGAATAACGTTCTTGCAGT ACACGTTTCAAGTATCTTATCGCTATAATAATGCCAAGAAAGACAGTGCACCTTGCAAAATAT AAAGCAGTTATCATCTTTATGTTGAACAAGATCAATAAACAGAAATCCTGAACAGATAGTTAGT TATTATAAGGCATGCAGAGATGACAGTGACCCAGTTGCTTCGGATGCAAGAGCTAAATTTG AGTTTCTTGGCGAAAAAGATAATGAATGTCAGCTGTACTCAGTTCAGATACGTTAAAAACGGA

		ACTAAAGTAAGACCGGATGAGGCTAAGGAAAAAGAAAGATTCAAAGCGATAATCGGTCTTT ATCTTACAGTCATGTACCTGATAACCAAGAATATGGTTTATATCAATTCAAGATATGTGACCG CATTTCATTGCCCTTGAAGGGACACGCAACTTCATGGTGTAAAGTTTATGATCAGAAGAACTG CAACCGAATCTTACAAAAAGTTTATTGATCCGAAAACTTGTGGTGACTATGGCCTCAGAAA TAATAAACGCGCAAGAACTTATATCGAGCAGAATATGGATAAGATGTCCAAGTGTACTAGTT ACTGGAATGAATACAGAAATGCCGTAGCACATCTTTGGTTATCAGGAATATGAATCAGTAT ATTAAAAATGTTAAGAATATAGGCAGTTGTTTTGAGCTGTATCATTATATTATGCAGCGTTTC CTGTTAGACAAAGAGAATATTGCTGAATCACTTAGAGAATATGATGATTTATATAAGAGAA GGGATGCTATAGAAAAGACTTCGTTAAGGCTTTGAATACTCGTTCGGATACAACCTTGCAA GATATAAGAAATCTTTCAATTGCAGAACTGTTTGACAGAAATGATACTGAGCTGGAGAGAACG AATAAGCTTAGAAATGAAGCAATCAAAGCAGATATAGAAGAAATCTGA
6	Cd15 amino acid sequence	MDKEKTTVEGKNTNQKSDVLKSLAKANGLKSSFVIGNEVMTSFGGRNSAILEKKITGSRIENLN PNVAFYVKKHISDSCNPDGKYDVKSKRMKEKAVVDDPVVYVSPKASNVHAGQDLIGCKNVLE ERYFGKTFDDNIHQLIYNILDEIKILAVHVSNAATFAINNILIEGKENEDFIGNLSVLNTFDEFENYE THPKFANKSAIKENLRKSKVFFDKIKKGNKLGYPGQAFYYATGTGKNLIFTKKSSETTYELLALVG SLRGFCVHDEVMDNKVKSRSWLYNAQKELKPDFLKADELYSKEVEKIDSDFVNNVTVDLHIIH DAIDVIDGSADWQKITNEYDFIIRKCFKNIGFSIKRLRETMIIEQMKVLGCKDCDRENCCKGYGKCF KNKKYDSVRSRLNRIVDFIIFRHYNDEAILKNVSLRLTCMSEEEKOKRFYLPKALWKKYNYVF RNYVLKKNLNGKSISGLKEKAIENSIDINSVKISLGDPPDYFCKFYLLTFFLDGKEINDLLTTLINKFDN IASFISVMKNDKLSIDCEVPEYSFFANSAQITSDLRVINSFARMQAPAEPSKDDMYRDALDILGM DDLSEDGKKQLEDTVLCRDENGKYMKKEDNNPKRDTNFRNFGNNVLASTRFKYLIYYNNNAKK TRALANNKAVIIFMLNKINKQNPQIVSYKACRDDSDPVASDAEAKIEFLAEKIMNVSCQTFRYV KNGTKVRPDEAKEKERFKAIIGLYLTVMYLITKNMVYINSRYVTAHFCLERDSELHGKFDQKKL QPNLTKKFIDPKTCGDYGLRNNKRARTYIEQNMDKMSNCTSYWNEYRNAVAHLSVIRNMNQYI KNVKNIGSCFELYHYIMQRFLLDKENIAESLREYDDFIKKKGCRYKDFVKALNTPFGYNLARYKN LSIAELFDRNDTELERTNKLNRNEAIKADIEI

[00129] Protein expression was performed using 200 ml of Terrific broth in a 1000cc flask. The E. coli cells harboring the plasmid were cultured at 37°C with shaking at 180 rpm. When the optical density of the culture reached between 0.6 and 0.8, production of the Cas13 enzymes was induced with 400 µM IPTG. The culture was then shifted to a lower temperature (16-18°C) and allowed to grow overnight to express the protein.

[00130] After the overnight induction, cells were harvested and resuspended in a lysis buffer containing 50 mM Tris-HCl (pH 7.0), 500 mM NaCl, 5% glycerol, 1 mM Tris(2-carboxyethyl)phosphine (TCEP), and 0.5 mM Phenylmethylsulfonyl fluoride (PMSF), supplemented with an EDTA-free protease inhibitor cocktail from Roche. To aid in cell disruption, lysozyme was added to the lysate at a concentration of 0.1-1 mg/ml, along with 500 mg to 1 g/L of protamine sulfate. This mixture was incubated on ice for 30 minutes with gentle swirling for homogeneous mixing.

[00131] The cells were subsequently lysed by sonication, performed on ice to prevent overheating, with settings adjusted to 60% amplitude, 0.5 cycle, and a pulse rate of 360 seconds on. Cell debris was then eliminated by centrifugation at 14,000 g for 30 minutes at 4°C, followed by filtration through a 0.45-micron filter.

[00132] The clarified cell lysate was processed through an affinity chromatography column loaded with Ni-NTA resin from Sigma-Aldrich at a proportion of 1cc resin per 10cc of cell lysate. The column was washed with a buffer containing 50 mM sodium

phosphate (pH 8.0), 300 mM NaCl, and 10 mM Imidazole. The Cas13 protein was then eluted using a buffer with elevated imidazole concentration (300 mM) and TCEP. The lysate, flow-through, wash, and elution fractions were all separately collected and assessed by SDS-PAGE for protein purity.

- 5 [00133] For protein concentration, fractions containing Cas13 were processed using Amicon filters with a 3k or 30k molecular weight cut-off. The concentrated enzyme was then incubated with TEV and EK proteases to remove the affinity tags. This step was performed at 4°C overnight while dialyzing against an ion-exchange buffer (50 mM Tris-HCl pH 7.0, 250 mM KCl, 5% glycerol, 1 mM TCEP) to facilitate tag cleavage.
- 10 [00134] The tag-free Cas13 enzymes were then applied to an Amicon Ultra-0.5ml centrifugal Filter Ultracel-50k (Merck Millipore) for final purification. The enzyme solution was finally equilibrated with a storage buffer optimized for enzyme stability and activity, which included 20 mM Tris-HCl (pH 7.0), 200 mM KCl, 5% glycerol, and 1 mM TCEP.
- 15 [00135] The final products, post-protease treatment and concentration, were verified for purity and integrity by SDS-PAGE analysis, confirming the successful expression and purification of the Cas13 enzyme suitable for downstream enzymatic assays.

[00136] **Example 2: Reaction Buffer Preparation**

- 20 [00137] Base Reaction Buffer was composed of 20 mM HEPES, 50 mM KCl, 5 mM MgCl₂, and 5% glycerol, pH=6.8. All salts, organic solvents, and proteins used in the experiment were of analytical grade and obtained from commercial sources (**Table 2**). These reagents were added to the Base Reaction Buffer in various combinations as described in the subsequent Examples. Each component was measured and mixed
- 25 thoroughly to ensure homogeneity of the buffer solutions.

[00138] **Table 2- The list of chemicals used in this study**

No	Name	CAS Number	Compony
1	Na acetate	127-09-3	Merck
2	Li ₂ SO ₄	1037-48-7	Merck
3	MgCl ₂	7786303	Sigma-Aldrich
4	KBr	7758-02-3	Merck
5	KSCN	333-20-0	Sigma-Aldrich
6	Na formate	141-53-7	Merck
7	Ca Acetate	62-54-4	Sigma-Aldrich
8	CdCl ₂	10108-64-2	Merck

9	NiCl ₂	7718-54-9	Merck
10	AlCl ₃	7446-70-0	Merck
11	CONO ₃	10026-22-9	Sigma-Aldrich
12	MgSO ₄	7487-88-9	Merck
13	CdSO ₄	10124-36-4	Sigma-Aldrich
14	MnSO ₄	10034-96-5	Merck
15	MnCl ₂	10034-96-5	Sigma-Aldrich
16	KCl	7447-40-7	Merck
17	Pb	32110-50-1	Sigma-Aldrich
18	Al ₂ (SO ₄) ₃	10043-01-3	Sigma-Aldrich
19	Ni(NO ₃) ₂	13478-00-7	Merck
20	ZnCl ₂	7646-85-7	Sigma-Aldrich
21	CaCl ₂	10043-52-4	Sigma-Aldrich
22	NaCl	7647-14-5	Merck
23	K ₂ SO ₄	7778-80-5	Sigma-Aldrich
21	FeSO ₄	7782-63-0	Merck
25	CuSO ₄	7758-98-7	Sigma-Aldrich
26	NaN ₃	26628-22-8	Merck
27	HgCl ₂	7487-94-7	Sigma-Aldrich
28	NiSO ₄	1010-97-0	Merck
29	Sodium citrate	6132-04-3	Sigma-Aldrich
30	(NH ₄) ₂ SO ₄	7783-20-2	Sigma-Aldrich
31	KH ₂ PO ₄	7778-77-0	Merck
32	CTAB	57-09-0	Sigma-Aldrich
33	Urea	57-13-6	Merck
34	EDTA	60-00-4	Sigma-Aldrich
35	Dithiothreitol (DTT)	3483-12-3	Merck
36	SDS	151-21-3	Merck
37	Ethanol	64-17-5	Sigma-Aldrich
38	Acetonitrile	75-05-8	Merck
39	Isobutanol	78-83-1	Sigma-Aldrich
40	Ethylene glycol	107-21-1	Merck
41	Phenylmethylsulfonyl fluoride (PMSF)	329-98-6	Sigma-Aldrich
42	Tween 20	9005-64-5	Merck
43	Methanol	67-56-1	Merck
44	Acetone	67-64-1	Merck
45	2-Propanol	67-63-0	Sigma-Aldrich
46	Beta-Mercaptoethanol	60-24-2	Merck
47	Iodoacetamide	144-48-9	Sigma-Aldrich
48	2-Methyl-2,4-pentanediol (MPD)	107415	Sigma-Aldrich
49	Butanediol	110-63-4	Merck
50	Casein	9000-71-9	Sigma-Aldrich

[00139] **Example 3: Nucleic acid preparation**

[00140] The ability of buffers of this invention to enhance the CRISPR-Cas13-Cd14 based detection was tested. To address this, the *in vitro* collateral cleavage activity of Cas13 enzymes were evaluated using an artificial target.

5 [00141] Artificial target sequence with either a C, G, A or U protospacer flanking sequence (PFS) were designed. DNA oligo templates for T7 transcription were synthesised and cloned in pUC57 by GenScript (**Table 3**).

[00142] **Table 3. DNA fragments for production of RNA substrates (i.e. target sequences)**

SEQ ID NO:	Name	Sequence
7	ssRNA 1 (C PFS)	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGT AGAACAGCAATCTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAG CTGTTTCCTGTGTTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAA G
8	ssRNA 1 (G PFS)	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGT AGAACAGCAATCTAGTTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAG CTGTTTCCTGTGTTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAA G
9	ssRNA 1 (A PFS)	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGT AGAACAGCAATCTAATTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAG CTGTTTCCTGTGTTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAA G
10	ssRNA 1 (U PFS)	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGT AGAACAGCAATCTATTTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAG CTGTTTCCTGTGTTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAA G

10

[00143] Phusion™ High-Fidelity PCR Master Mix (2X) (Thermo Fisher Scientific) was used to amplify the template DNA with forward primer (TAATACGACTCACTATAG) (SEQ ID NO: **11**) and reverse (CTTTATGCTTCCGGCTCG) primer (SEQ ID NO: **12**). The PCR reaction was
15 assembled in a 25-μL volume following the manufacturer’s instructions, using cycling conditions recommended by the supplier, with an annealing temperature of 55°C and 35 cycles. The integrity of the amplified sequence was confirmed by Sanger sequencing. The PCR product was analyzed on a 2% agarose gel, and the target band was extracted using the GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific). To verify successful
20 recovery, 3 μL of the purified DNA was run on a 2% agarose gel. The DNA concentration was measured using a Picodrop and Qubit Fluorometer 4.

[00144] For single-stranded RNA (ssRNA) synthesis, the TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific) was used, with the T7 RNA polymerase

forward primer facilitating transcription. The IVT reaction was set up at room temperature according to the manufacturer's protocol, using 1 µg of template DNA, followed by thorough mixing and brief centrifugation. The reaction was incubated at 37°C for 4 hours in a water bath.

- 5 [00145] To prevent template DNA contamination in downstream applications, DNase I digestion was performed immediately after the IVT reaction. 1 µL of DNase I (1 U/µL, included in the kit) was added to the reaction mix and incubated at 37°C for 15 minutes.
- [00146] For RNA analysis, 0.5 µL of the IVT product was diluted in 10 µL of DEPC-treated water. Then, 10 µL of this diluted RNA was mixed with 10 µL of 2X RNA
- 10 Loading Dye Solution (provided in the kit), incubated at 70°C for 10 minutes, and then chilled on ice before loading onto a 2% agarose gel. The RNA samples were analyzed against an RNA ladder for size verification.

[00147] RNA purification from IVT reaction was performed using GeneJET RNA Cleanup and Concentration kit (Thermo Fisher Scientific) as instructed by manufacturer.

- 15 The purified RNA was stored at -80°C until use. 0.5 µL of the purified IVT product was diluted in 10 µL of DEPC-treated water and 10 µL of the diluted sample was mixed with 10 µL of 2X RNA Loading Dye Solution and was heated the sample at 70°C for 10 minutes and was chilled on ice prior to loading on a 2% Agarose Gel against an RNA Ladder.

20

[00148] **Example 4. Ionic Influence on Cas13-Cd14 Activity under Varying Salt Conditions**

- [00149] The activity of Cas13-Cd14, similar to other enzymes, is known to be influenced by various factors, including the ionic environment in which it operates. Ions
- 25 can affect the folding, stability, and catalytic activity of RNA and proteins by altering the electrostatic interactions and the structural conformation of biomolecules. Therefore, understanding the impact of different ionic conditions on Cas13-Cd14 activity is crucial for optimizing its performance in various applications.

- [00150] In this study, the aim was to investigate how the addition of various salts to the
- 30 reaction buffer alters the activity of Cas13-Cd14. The rationale behind this examination is rooted in the premise that ions play a significant role in enzymatic activity and may either enhance or inhibit the catalytic function of Cas13d. By systematically varying the

type and concentration of salts in the reaction buffer, it was aimed to delineate the optimal conditions that support the enzymatic activity of Cas13-Cd14.

[00151] **Methods**

[00152] The base reaction buffer was composed of 20 mM HEPES, 50 mM KCl, 5 mM
5 MgCl₂, and 5% glycerol, pH=6.8. This buffer served as the control environment for all subsequent experiments.

[00153] A range of 10, 20, 40, and 60 mM of salts including KH₂PO₄, CdSO₄,
Co(NO₃)₂, HCOONa, Ca(C₂H₃O₂)₂, CH₃COONa, (NH₄)₂SO₄, AlCl₃, KSCN, FeSO₄,
CuSO₄, LiSO₄, Al₂(SO₄)₃, Ni(NO₃)₂, ZnCl₂, CaCl₂, NaCl, and K₂SO₄ were added to the
10 base reaction buffer. The final concentrations of the salts were varied in a systematic manner to assess the influence of ionic strength and salt composition on Cas13-Cd14 activity.

[00154] For collateral cleavage assay, a 20μL detection system was prepared that consisted of 100 nM Cas13-Cd14, 100 nM crRNA
15 (CAACUACAACCCUGUCAAAUACAGGGUUCUGAAACUAGAUUGCUGUUC UACCAAGUAAUCCA) (SEQ ID NO: 13), 250nM ssRNA reporter (/56-FAM/rUrUrUrU/3IABkFQ/), and 250nM target ssRNA ((an equal mixture of the four targets listed in Table 3) in a 1× cleavage buffer. Fluorescence readouts were taken in 1 min intervals for a total of 60 min on the ABI Real-time PCR (Applied Biosystems, CA,
20 USA). The fluorescence signal, indicative of Cas13-Cd14 activity, was recorded at regular intervals for a period of 1 hour. Since the enzyme activity for each test reached to its peak before 5 minutes, the activity of enzyme at 5 minute is shown in the graphs as means ± SD (n = 3). To facilitate comparisons across varying experimental conditions, fluorescence for background conditions (no target ssRNA) were subtracted from samples
25 to generate background subtracted fluorescence.

[00155] **Results**

[00156] The addition of different salts to the reaction buffer resulted in varied effects on Cas13-Cd14 activity, as shown in Figure 1. KH₂PO₄, HCOONa, Ca(C₂H₃O₂)₂, CH₃COONa, (NH₄)₂SO₄, KSCN, CaCl₂, and NaCl enhanced the activity of Cas13-Cd14,
30 while CdSO₄, Co(NO₃)₂, AlCl₃, CuSO₄, LiSO₄, Al₂(SO₄)₃, Ni(NO₃)₂, ZnCl₂, FeSO₄, and K₂SO₄ inhibited the activity of enzyme. The data indicate a clear correlation between salt composition and Cas13-Cd14 activity.

[00157] The addition of potassium thiocyanate (KSCN), sodium formate (HCOONa), monopotassium phosphate (KH_2PO_4), sodium acetate (CH_3COONa), calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) resulted in an impressive 2.70-, 2.67-, 2.24-, 2.21-, 2.03-, and 1.88-fold increase in Cas13-Cd14 activity, respectively.

- 5 [00158] In summary, the present results demonstrated that Cas13 activity was sensitive to the ionic composition of the reaction buffer. Both enhancing and inhibiting effects were observed depending on the type and concentration of the salt added. These findings could inform the optimization of reaction conditions for Cas13-based applications.

10 [00159] **Example 5: The Influence of Organic Compounds on Cas13-Cd14 Activity**

[00160] This experiment was designed to assess the influence of different organic compounds at variable concentrations on the enzymatic activity of Cas13-Cd14, providing insight into the optimal conditions for its function and potential regulatory mechanisms.

15 [00161] **Methods**

[00162] The base reaction buffer was as described in Example 3. A set of organic compounds was prepared at varying concentrations. Each compound was added to the reaction mix to achieve the final testing concentrations. A control without any added compounds was also maintained.

- 20 [00163] Fluorescence signals were monitored at regular intervals over an hour to measure Cas13d activity. As peak enzyme activity was achieved within 5 minutes in each assay, the graphs present the activity levels at the 5-minute mark as mean values with their standard deviations (means $\pm$ SD; n = 3). Baseline activity was established using the control reaction mix.

25 [00164] **Results**

[00165] It was observed that certain organic compounds enhanced Cas13-Cd14 activity at different concentrations (Figure 2). It was observed enhanced activity of Cas13-Cd14 by introduction of Tween 20, Dithiothreitol (DTT), Phenylmethylsulfonyl fluoride (PMSF), 2-Methyl-2,4-pentanediol (MPD), and acetone. Some compounds such as
30 EDTA and Urea did not exhibit a significant impact on enzymatic activity when compared with the control.

[00166] **Example 6. Assessing the impact of Casein on Cas13-Cd14 Activity**

[00167] Adding a protein to an enzyme's reaction buffer may enhance the enzyme's activity through various mechanisms. The added protein can act as a cofactor, facilitating the enzyme's ability to bind to its substrate or aiding in the catalytic process. It might also
5 stabilize the enzyme's structure, maintaining it in an optimal conformation for activity or protecting it from denaturation and degradation. In some cases, the protein may interact with the enzyme to form a complex that has greater activity than the enzyme alone, or it might block the action of inhibitors, thereby sustaining the enzyme's function. Here the strategy of adding proteins to Cas13-Cd14 reaction buffers to modulate enzyme activities
10 in biochemical assays was examined. Given the lack of existing data on the relationship between casein and Cas13-Cd14, the aim was to elucidate the potential modulatory effects of casein on the collateral cleavage activity of Cas13-Cd14.

[00168] **Methods**

[00169] To investigate the influence of Casein on Cas13-Cd14, casein with the concentration of 0.005, 0.01, 0.03, and 0.05 mg/ml were added to control reaction buffer.
15 The experiment and data analysis were performed as explained in Example 4.

[00170] **Results**

[00171] Incorporation of casein into the Cas13-Cd14 reaction system revealed a notable concentration-dependent enhancement of enzymatic activity (Figure 3). Without casein,
20 Cas13-Cd14 displayed a baseline activity with an average background subtracted fluorescence intensity of 108K. Upon introducing 0.005 mg/ml of casein, a 1.9-fold increase in enzymatic activity was observed. Further increase in casein concentration to 0.01 and 0.03 mg/ml resulted in 2.8- and 3-fold increases in fluorescence intensities, respectively, over the control. The signal intensity slightly declined and showed 1.2-fold
25 increase compared the control when the concentration of casein increased to 0.05 mg/ml. These findings suggested a stimulatory role for casein in the context of Cas13-Cd14 activity.

[00172] **Example 7. Ionic Influence on Cas13-Cd14 Activity in SARS-CoV-2**

30 **Detection**

[00173] The ability of buffers of this invention to enhance the SARS-CoV-2 detection using CRISPR-Cas13-Cd14 was tested.

[00174] **Methods**

[00175] A segment of the SARS-CoV-2 N gene (**Table 4**) (Broughton JP et al.'s study on CRISPR-Cas12-based detection of SARS-CoV-2 (Nat Biotechnol. 2020 Jul;38(7):870-874), designated as the target, was artificially synthesized and then inserted into the pUC57 vector by GenScript. This cloned fragment served as the template for subsequent T7 in-vitro transcription (IVT) to generate single-stranded RNA (ssRNA) templates. Single-stranded RNA (ssRNA) preparation and analysis were performed as described in Example 3.

[00176] **Table 4. Sense DNA template of SARS-CoV-2 N gene for production of RNA substrates (i.e. target sequences)**

SEQ ID NO	Name	Sequence
14	Fragment of SARS-CoV-2 N gene	5'- CCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGAC GGTAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAAC TGGGCCAGAAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATA TGGGTTGCAACTGAGGGAGCCTTGAATACACCAAAGATCACATTGGCA CCCGCAATCCTGCTAACAAATGCTGCAATCGTGCTACAACCTTCTCAAGGA ACAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGGCGGCAGT CAAGCCTCTTCTCGTTCTCATCAGTACGTAGTCGCAACAGTTCAAGAAATTC AACTCCAGGCAGCAGTAGGGGAACCTTCTCTGCTAGAAATGGCTGGCAAT GGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCGC TTGAGAGCAAAATGTCTGGTAAAGGCCAACAAACAAGGCCAAACTGT CACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCCAAAACGT ACTGCCACTAAGCATACAATGTAACACAAGCTTTCGGCAGACGTGGTC CAGAACAAACCAAGGAAATTTTGGGGACCAAGGAATTAATCAGACAAGG AACTGATTACAAACATTGGCCGCAATTGCACAATTTGCCCCAGCGCTT CAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTCACACCTTCGGG AACGTGGTTGACCTACACAGGTGCCATCAAAATGGATGACAAAAGATCCAA ATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAA CATTCACCAACAGAGCCTAAAAAGGACAAAAGAAAGAGGCTGATGA AACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACTGTG-3'

[00177] The base reaction buffer consisted of 20 mM HEPES, 50 mM KCl, 5 mM MgCl₂, and 5% glycerol (pH 6.8), serving as the control environment for all subsequent experiments. To evaluate the impact of ionic strength and salt composition on Cas13-
Cd14 activity, varying concentrations (0.25, 0.5, 1, 2.5, 5 and 10 mM) of Li₂SO₄, Al₂(SO₄)₃, Ni(NO₃)₂, ZnCl₂, CaCl₂, and NaCl were systematically added to the base reaction buffer. Since most of the salts tested in the previous experiment (Example 4) exhibited the highest enhancement of activity at lower concentrations (10 mM), a range of salt concentrations from 0.25 mM to 10 mM was examined in this experiment.

[00178] For the collateral cleavage assay, a 20 µL detection system was prepared, containing 100 nM Cas13-Cd14, 50 nM crRNA (**Table 5**), 250 nM ssRNA reporter (/56-FAM/rUrUrUrUrU/3IABkFQ/), and 250 nM target ssRNA (an equal mixture of the four

targets listed in **Table 3**) in a 1× cleavage buffer. Fluorescence signals were recorded at 1-minute intervals for a total of 60 minutes using an ABI Real-Time PCR system (Applied Biosystems, CA, USA). Since enzyme activity peaked within the first 5 minutes, fluorescence measurements at the 5-minute mark are presented in the graphs as means ± SD (n=3). To ensure consistency across experimental conditions, background fluorescence (from reactions without target ssRNA) was subtracted from all samples to generate background-corrected fluorescence values.

[00179] **Table 5.** crRNA sequences (5' to 3') for SARS-CoV-2 detection.

SEQ ID NO: 15	Cd14-CoV crRNA	GCAACUACAACCCUGUCAAAUACAGGGUUCUGA AACUUGUGCAAUUUGCGGCCAAUGUUU
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[00180] **Results**

[00181] The addition of different salts to the reaction buffer resulted in varied effects on Cas13-Cd14 activity. Ni(NO₃)₂, LiSO₄, CaCl₂, and NaCl enhanced the activity of Cas13-Cd14, while Al₂(SO₄)₃ and ZnCl₂ inhibited the activity of enzyme. The data indicate a clear correlation between salt composition and Cas13-Cd14 activity (Figure 4).

[00182] The addition of Ni(NO₃)₂, LiSO₄, CaCl₂, and NaCl resulted in an impressive 3.9-, 2.6-, 4.0-, and 2.9-fold increase in Cas13-Cd14 activity, respectively.

[00183] In summary, the present results demonstrated that Cas13-Cd14-based disease detection can be optimized by modulating the ionic composition of the reaction buffer. The effects varied depending on the type and concentration of the salt, with both enhancement and inhibition observed. Notably, Ni(NO₃)₂ enhanced Cas13-Cd14 activity at lower concentrations, suggesting that nickel ions may play a role in stabilizing the enzyme's active conformation, facilitating RNA binding, or improving the interaction between the enzyme and its crRNA-target complex. However, at higher concentrations, potential inhibitory effects may arise due to enzyme structural perturbation or non-specific ionic interactions interfering with the cleavage mechanism.

[00184] **Example 8. The Influence of Organic Compounds on Cas13-Cd14 activity in SARS-CoV-2 detection**

[00185] This experiment aimed to evaluate the impact of various organic compounds at different concentrations on the Cas13-Cd14 base SARS-CoV-2 detection.

[00186] **Methods**

[00187] The base reaction buffer was prepared as described in Example 7. A range of organic compounds (DMSO, Tween 20, MPD, PMSF, propanol, acetone, EDTA, Urea, ethylene glycol, and methanol) was added to the reaction mix at varying concentrations, with a control reaction maintained without any additional compounds.

5 [00188] Collateral cleavage assay was performed as Example 7. Fluorescence signals were monitored at regular intervals over an hour to assess Cas13-Cd14 activity. Since peak activity was consistently observed within the first 5 minutes, the data presented in the graphs reflect the enzyme activity at the 5-minute mark as mean values with standard deviations (mean $\pm$ SD; n = 3). Baseline activity was established using the control reaction
10 mix.

[00189] **Results**

[00190] Organic compounds generally enhanced Cas13-Cd14 activity for SARS-CoV-2 detection (Figure 5). Notable enhancements were observed with DMSO, Tween 20, and ethylene glycol, which increased Cas13-Cd14 activity by up to 6.7, 5.8, and 5.5 times,
15 respectively. Additionally, MPD, PMSF, propanol, acetone, EDTA, urea, and methanol also significantly enhanced enzyme activity by up to 3.6, 2.6, 3.5, 3.2, 3.5, 2.2, and 3.9 times, respectively.

[00191] The effect of these organic compounds was concentration-dependent: while PMSF and DMSO increased Cas13-Cd14 activity at higher concentrations, compounds
20 such as Tween 20, propanol, methanol, and EDTA enhanced the activity at lower concentrations. In contrast, ethylene glycol significantly altered Cas13-Cd14 activity at mid-range concentrations.

[00192] **Example 9. The impact of Casein on Cas13-Cd14 activity in SARS-CoV-2
25 detection**

[00193] Here the strategy of adding casein to Cas13-Cd14 reaction buffers to enhance the activity of enzyme for SARS-CoV-2 detection was examined.

[00194] **Methods**

[00195] To investigate the influence of Casein on Cas13-Cd14 based SARS-CoV-2
30 detection, casein with the concentration of 0.0015, 0.003, 0.006, 0.015, and 0.03, and 0.06 mg/ml were added to control reaction buffer. The experiment and data analysis were performed as explained in Example 4.

[00196] **Results**

[00197] Incorporation of casein into the Cas13-Cd14 reaction system revealed a notable concentration-dependent enhancement of enzymatic activity (Figure 6). Without casein, Cas13-Cd14 displayed a baseline activity with an average background subtracted
 5 fluorescence intensity of around 38K. Upon introducing 0.015 mg/ml of casein, 6.3-fold increase in enzymatic activity was observed. Further increases in casein concentration did not result in additional fluorescence intensity beyond 0.015 mg/mL of casein. These findings suggest a stimulatory role for casein in the context of Cas13-Cd14 activity.

10 [00198] **Example 10. Limit of Detection (LOD) Assay**

[00199] The limit of detection (LOD) is a critical evaluation of CRISPR-Cas13 sensitivity in identifying target nucleic acids. This assessment highlights Cas13-Cd14 enzyme's potential for sensitive SARS-CoV-2 detection diagnostics. To enhance the sensitivity of enzyme detection, casein was added to buffer.

15 [00200] **Methods**

[00201] In this experiment, the N-gene of SARS-CoV-2 was selected as the target sequence. Primers were designed using Oligo7 software, incorporating a T7 promoter sequence in the forward primer to enable in vitro transcription (IVT).

[00202] The target plasmid (pUC19+N-Gene) was synthesized by GenScript. Plasmid
 20 concentration was measured using an Invitrogen Qubit 4 Fluorometer, following the manufacturer's instructions. The DNA copy number per microliter was calculated using the formula:

[00203]
$$\text{DNA copy number per } \mu\text{L} = [(6.02 \times 10^{23}) \times (\text{plasmid concentration in ng}/\mu\text{L}) \times 10^{-9}] / [(\text{fragment length in nucleotides}) \times 660]$$

25 [00204] The plasmid was serially diluted from 0 to 1×10^3 copies/ μL , with the following concentrations: negative control (0), 1, 5, 10, 100, and 1×10^3 copies/ μL . The sensitivity of the RPA reaction was assessed using each dilution as a template, while RNase-free water served as a negative control.

[00205] The RPA reaction was conducted in a 50 μL final volume, with plasmid DNA
 30 concentrations ranging from 20 copies/ μL to 0.02 copies/ μL , based on the dilution of the plasmid stock and the addition of 1 μL of plasmid to the reaction. The reaction mixture contained 2.4 μL of each forward and reverse RPA primer (10 μM) (Table 6), 29.5 μL

primer-free rehydration buffer, 2.5 mM MgOAc, 1 µL target DNA, and RNase-free water to bring the total volume to 50 µL. The reaction was incubated in a 39°C water bath for 60 minutes.

5 [00206] **Table 6.** RPA Primer sequences (5' to 3')

Gene	SEQ		Sequence (5' to 3')
	ID NO:	Name	
N-Gene	16	F	AATTCTAATACGACTCACTATAGGGATGTAACA CAAGCTTTCGGCAGACGTGGTC
	17	R	CCAATTTGATGGCACCTGTGTAGGTCAACC

[00207] Following RPA, IVT was performed to generate RNA from the amplified DNA. The IVT reaction had a total volume of 20 µL and contained 8 µL ATP/CTP/GTP/UTP mix, 4 µL 5X TranscriptAid Reaction Buffer, 2 µL TranscriptAid Enzyme Mix, and 6 µL of RPA product. The mixture was incubated in a 37°C water bath
10 for 60 minutes.

[00208] The Cas13-Cd14 collateral cleavage assay was performed in a 20 µL reaction volume, with three replicates for both positive and negative samples. The reaction mixture included 4 µL of 5X cleavage buffer, 0.015 mg/ml casein, 200 nM 264 Cas13 enzyme, 200 nM crRNA, 250 nM ssRNA fluorescence probe (/56-FAM/rUrUrUrUrU/3IABkFQ/),
15 0.25 µL IVT product (for the positive reaction), 1 µL DEPC-treated water (for the negative control), and RNase-free water to reach a total volume of 20 µL.

[00209] Real-time fluorescence measurements were collected on Step One ABI Real-time PCR (Applied Biosystems, CA, USA) at 1 min intervals for a total of 60 min. To allow comparisons between different conditions, fluorescence for background conditions
20 (no target) were subtracted from samples to generate background subtracted fluorescence. The measurement of real-time background subtracted fluorescence output are shown in the graphs as means (n = 3).

[00210] **Results**

[00211] The sensitivity RPA amplification and IVT coupled with combination of
25 Cas13-Cd14 enzyme detection was tested here using a series of diluted positive quality controls (Figures 7).

[00212] The effective DNA copy number available in the Cas13-Cd14 collateral cleavage reaction was determined by accounting for dilution factors across both the RPA

and IVT steps. Starting with the initial DNA copies in the RPA reaction, 6 μL of the 50 μL RPA product was used for IVT. Following IVT, 1 μL of the 20 μL IVT product was transferred to the Cas13 reaction. The final effective DNA copies in the Cas13 assay were calculated using the formula:

5 [00213] $\text{Effective DNA copies} = \text{Initial DNA copies in RPA} \times 6/50 \times 0.25/20$

[00214] Through these sequential steps—RPA amplification, IVT conversion to RNA, and subsequent dilution into the Cas13 reaction—the effective DNA copies reaching Cas13 were calculated as follows: for initial DNA inputs of 1, 5, 10, 100, and 1000 copies in RPA, the final effective copies in Cas13 were 0.0015, 0.0075, 0.015, 0.15, and 1.5, respectively.

[00215] Cas13-Cd14 demonstrated strong sensitivity, with fluorescence signals at 0.006 copies/ μL significantly higher than the background (0 copies/ μL). This highlights its ability to reliably detect very low concentration of DNA.

15 [00216] **Example 11. The Influence of compounds on Cas13-Cd13 and Cas13-Cd15 activity in SARS-CoV-2 detection**

[00217] This experiment aimed to assess the effect of three compounds at varying concentrations on two different Cas13 enzymes, **Cas13-Cd13** and **Cas13-Cd15**, for SARS-CoV-2 detection. The goal was to determine whether the enhancement observed with these compounds in one enzyme could be replicated in others.

[00218] **Methods**

[00219] The base reaction buffer was prepared as described in Example 7. The best concentration of Tween 20 (0.25% V/V), MPD (2.5% V/V), and casein (0.015 mg/ml) observed in Examples 8 and 9 were added to the reaction mix, with a control reaction maintained without any additional compounds.

[00220] For the collateral cleavage assay, a 20 μL detection system was prepared, containing 200 nM Cas13-Cd13 or Cas13-Cd15, 200 nM crRNA (**Table 7**), 250 nM ssRNA reporter (/56-FAM/rArArArArA/3IABkFQ/), and 250 nM target ssRNA in a 1 $\times$ cleavage buffer. Fluorescence signals were recorded at 1-minute intervals for a total of 60 minutes using an ABI Real-Time PCR system (Applied Biosystems, CA, USA). Since enzyme activity peaked within the first 5 minutes, fluorescence measurements at the 5-minute mark are presented in the graphs as means $\pm$ SD (n = 3).

[00221] Fluorescence signals were monitored at regular intervals over an hour to assess Cas13-Cd14 activity. Since peak activity was consistently observed within the first 5 minutes, the data presented in the graphs reflect the enzyme activity at the 5-minute mark as mean values with standard deviations (mean $\pm$ SD; n = 3). Baseline activity was established using the control reaction mix.

[00222] **Table 7. crRNA sequences (5' to 3') for for SARS-CoV-2 detection.**

SEQ ID NO:	Name	Sequence
18	Cd13-CoV crRNA	GAAACGUACUACACCCUUUUUGUAAGGGUCUGAAACC CCGAAGAACGCUGAAGCGCUGGGG
19	Cd15-CoV crRNA	GAUCUAUAACCCUGCAUUUAUGUAGGGCUCUAAAACU UGUGCAAUUUGCGGCCAAUGUUU

[00223] **Results**

[00224] To determine whether the tested compounds could also enhance the activity of other Cas13 enzymes, the effects of Tween 20, MPD, and Casein on two additional Cas13 enzymes, Cas13-Cd13 and Cas13-Cd15 were examined. The present results showed that all three compounds significantly enhanced the activity of these enzymes (Figure 8). Notably, Tween 20 and Casein led to remarkable activity increases in Cas13-Cd15, enhancing its fluorescence signal by 10.4-fold and 11.2-fold, respectively. These findings suggest that the enhancement effect of these compounds is not limited to a single Cas13 variant but may be broadly applicable across different members of the Cas13 family, potentially improving the sensitivity and efficiency of CRISPR-Cas13-based detection systems.

[00225] The present disclosure has focused on examining the influence of various compounds on the activity of a singular Cas13d enzyme. However, the scope of the present inquiry extends beyond this specific interaction. It is essential to consider that these compounds may also affect the activity of other enzymes in the Cas13 family, each of which could respond differently to the same molecular interventions. Furthermore, the potential impact of these compounds on Cas12 enzymes should not be overlooked, as Cas12 enzymes possess collateral activities that are distinct from those of Cas13 enzymes. These collateral activities are crucial for the CRISPR system's function in diagnostics. While the present experimental data currently demonstrate enhancement of Cas13 enzymatic activity through the addition of novel buffer components, it can be reasonably anticipated that similar improvements may extend to Cas12 enzymes. This expectation is based on several shared mechanistic and structural features between Cas13 and Cas12

systems. Both enzymes are RNA-guided nucleases that require precise buffer conditions to maintain optimal tertiary conformation and catalytic activity. Furthermore, the identified compounds act through mechanisms such as stabilizing protein-RNA interactions, enhancing target binding, or facilitating ion-dependent conformational changes—features that are also critical for Cas12 function. Given these mechanistic similarities, and the lack of reported data refuting such effects on Cas12, it is plausible that these novel buffer additives will likewise enhance Cas12 activity. This potential cross-applicability supports the broader utility of the present buffer system across the CRISPR-Cas enzyme family.

References:

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- Xu J, Ma J, Li Y, Kang L, Yuan B, Li S, Chao J, Wang L, Wang J, Su S, Yuan Y. A general RPA-CRISPR/Cas12a sensing platform for Brucella spp. detection in blood and milk samples. Sensors and Actuators B: Chemical. 2022 Aug 1;364:131864.

Claims

1. A buffer for enhancing enzymatic cleavage activity of a Cas protein, comprising one or more components selected from the group consisting of:
 - a salt selected from the group consisting of monopotassium phosphate (KH_2PO_4), sodium formate (HCOONa), calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$), sodium acetate (CH_3COONa), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), potassium thiocyanate (KSCN), calcium chloride (CaCl_2), sodium chloride (NaCl), Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$), lithium sulfate (LiSO_4);
 - an organic solvent selected from the group consisting of Tween 20, Dithiothreitol (DTT), Phenylmethylsulfonyl fluoride (PMSF), 2-Methyl-2,4-pentanediol (MPD), acetone, dimethylsulfoxide (DMSO), ethylene glycol, methanol, 2-propanol, EDTA, urea; and
 - a protein which is casein.
2. The buffer of claim 1, further comprising a base composition comprising HEPES, KCl, MgCl_2 , and glycerol.
3. The buffer of claim 2, wherein the base composition is one of the following:
 - a base composition comprising about 20 mM HEPES, about 50 mM KCl, about 5 mM MgCl_2 , about 5% glycerol, and having a pH of about 6.8;
 - a base composition comprising about 20 mM HEPES, about 50 mM NaCl, about 10 mM MgCl_2 , about 5% glycerol, and having a pH of about 7.0;
 - a base composition comprising about 20 mM HEPES, about 50 mM NaCl, about 10 mM MgCl_2 , about 5% glycerol, about 1 $\mu\text{g/ml}$ BSA, and having a pH of about 7.0;
 - a base composition comprising about 20 mM HEPES, about 60 mM NaCl, about 6 mM MgCl_2 , about 5% glycerol, and having a pH of about 6.8;
 - a base composition comprising about 20 mM HEPES, about 60 mM NaCl, about 5% PEG, about 5 μM DTT, and having a pH of about 6.8; and

a base composition comprising about 20 mM HEPES, about 60 mM KCl, about 5% PEG, and having a pH of about pH 8.0.

4. The buffer of claim 1, wherein the Cas protein is selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c, Cas12a, Cas12b, Cas12c, Cas12d,
5 and Cas12f, optionally the Cas13d protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4, and 6.
5. The buffer of claim 1, wherein the concentration of each of the salt is 5 mM - 3 M, 10-60 mM, or 0.25-10 mM.
6. The buffer of claim 1, wherein the concentration of each of the organic solvent is
10 5 mM - 3 M, or 1-5 mM.
7. The buffer of claim 1, wherein the concentration of monopotassium phosphate (KH_2PO_4) is 10-60 mM.
8. The buffer of claim 1, wherein the concentration of sodium formate (HCOONa) is 10-60 mM.
9. The buffer of claim 1, wherein the concentration of calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$)
15 is 10-60 mM.
10. The buffer of claim 1, wherein the concentration of sodium acetate (CH_3COONa) is 10-60 mM.
11. The buffer of claim 1, wherein the concentration of ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) is 10-60 mM.
20
12. The buffer of claim 1, wherein the concentration of potassium thiocyanate (KSCN) is 10-60 mM.
13. The buffer of claim 1, wherein the concentration of calcium chloride (CaCl_2) is 10-60 mM, or 1.25-50 mM.
14. The buffer of claim 1, wherein the concentration of sodium chloride (NaCl) is
25 0.25-10 mM, or 1.25-50 mM.

15. The buffer of claim 1, wherein the concentration of Nickel(II) nitrate ($\text{Ni}(\text{NO}_3)_2$) is 0.25-10 mM.
16. The buffer of claim 1, wherein the concentration of lithium sulfate (LiSO_4) is 0.25-10 mM.
- 5 17. The buffer of claim 1, wherein the concentration of DTT is 0.1-15 mM, or 1-5 mM.
18. The buffer of claim 1, wherein the concentration of PMSF is 0.1-15 mM, 0.25-10 mM, or 1-2.5 mM.
- 10 19. The buffer of claim 1, wherein the concentration of DMSO is 0.1-15 mM, 0.25-10 mM, or 1-2.5 mM.
20. The buffer of claim 1, wherein the concentration of Tween 20 is 0.1-15 mM, 0.25-10 mM, or 2.5-5 mM.
21. The buffer of claim 1, wherein the concentration of ethylene glycol is 0.1-15 mM, 0.25-10 mM, or about 2.5 mM.
- 15 22. The buffer of claim 1, wherein the concentration of methanol is 0.1-10 mM, 0.25-5 mM, or 1-5 mM.
23. The buffer of claim 1, wherein the concentration of 2-propanol is 0.1-15 mM, 0.25-10 mM, or 1-2.5 mM.
24. The buffer of claim 1, wherein the concentration of acetone is 0.1-15 mM, 0.25-10 mM, or 1-2.5 mM.
- 20 25. The buffer of claim 1, wherein the concentration of MDP is 0.1-15 mM, 0.25-10 mM, or about 4 mM.
26. The buffer of claim 1, wherein the concentration of EDTA is 0.1-5 mM, or 0.25-1 mM.

27. The buffer of claim 1, wherein the concentration of urea is 0.1-15 mM, 0.25-10 mM.
28. The buffer of claim 1, wherein the concentration of casein is 0.001-10 mg/mL, or 0.01-1 mg/mL.
- 5 29. The buffer of claim 28, wherein the concentration of casein is 0.005-0.05 mg/mL, or 0.0015-0.06 mg/mL.
30. The buffer of any one of claims 1-29, wherein the buffer has a PH of between 6.0 and 8.0.
- 10 31. The buffer of any one of claims 1-30, wherein the enzymatic cleavage activity is increased by 1.5-20 fold.
32. The buffer of claim 31, wherein the enzymatic cleavage activity is increased by 1.76-3.04 fold, or 1.88-2.70 fold, or 10.4-11.2 fold.
33. The buffer of claim 2, wherein the buffer comprises the base composition and one or more salts of claim 1.
- 15 34. The buffer to claim 2, wherein the buffer comprises the base composition and one or more organic solvents of claim 1.
35. The buffer to claim 2, wherein the buffer comprises the base composition and casein.
- 20 36. The buffer to claim 2, wherein the buffer comprises the base composition, one or more salts and one or more organic solvents of claim 1.
37. The buffer to claim 2, wherein the buffer comprises the base composition, one or more salts of claim 1, and casein.
38. The buffer to claim 2, wherein the buffer comprises the base composition, one or more organic solvents of claim 1, and casein.

39. The buffer to claim 2, wherein the buffer comprises the base composition, one or more salts of claim 1, one or more organic solvents of claim 1, and casein.
40. A detection kit for a nucleic acid, comprising:
- (a) the buffer of any one of claims 1-39;
 - 5 (b) a Cas protein selected from the group consisting of Cas13d, Cas13a, Cas13b, Cas13c, Cas12a, Cas12b, Cas12c, Cas12d, and Cas12f; and
 - (c) a crRNA configured to target the nucleic acid and form a complex with the Cas protein.
41. The detection kit of claim 40, further comprising a detecting agent comprising a
10 probe nucleic acid, wherein the detecting agent generates a detectable signal when the probe nucleic acid is cleaved by the Cas protein.
42. The detection kit of claim 41, wherein the probe nucleic acid is a fluorescent probe, wherein the 5' end of the fluorescent probe sequence is labeled with a fluorescent group, and the 3' end is labeled with a quenching group; or a
15 colorimetric probe.
43. The detection kit of claim 42, wherein the fluorescent group is selected from the group consisting of FAM, VIC, HEX, TRT, Cy3, Cy5, ROX, JOE and Texas Red, and the quenching group is selected from the group consisting of DABCYL, MGB, BHQ-1, BHQ-2 and BHQ-3.
- 20 44. The detection kit of claim 42, wherein the colorimetric probe comprises FAM and Biotin, and is for detection by a lateral flow strip.
45. The detection kit of any one of claims 40-44, wherein the Cas protein and the crRNA are in the form of a ribonucleoprotein complex.
- 25 46. The detection kit of any one of claims 40-45, wherein the Cas protein and the crRNA are lyophilized.
47. The detection kit of any one of claims 40-46, wherein shelf-life of the Cas protein and the crRNA is increased by 30-300% in the presence of the buffer of any one

of claims 1-39, compared to the self-life of the Cas protein and the crRNA in the absence of the buffer of any one of claims 1-39.

48. A method of detecting a nucleic acid in a sample by clustered regularly interspaced short palindromic repeats and CRISPR associated protein (CRISPR-cas), using the detection kit of any one of claims 40-47, the method comprising:

incubating the sample containing the nucleic acid with the Cas protein and the crRNA, wherein when the nucleic acid is present in the sample, enzymatic cleavage activity of the Cas protein is induced to generate a detectable signal when the probe nucleic acid is cleaved; wherein the buffer enhances the enzymatic cleavage activity of the Cas protein.

49. The method of claim 48, further comprising amplifying the nucleic acid in the sample, using a technology selected from the group consisting of Polymerase Chain Reaction (PCR), Recombinase Polymerase Amplification (RPA), Loop-Mediated Isothermal Amplification (LAMP), and Rolling Circle Amplification (RCA).

50. The method of claim 48, wherein the method does not comprise amplifying the nucleic acid in the sample.

51. The method of claim 48, further comprising using a detection method selected from the group consisting of a colorimetric detection method, a fluorescent detection method and an electrochemical detection method to detect the detectable signal which is a colorimetric signal, a fluorescent signal, or an electrochemical signal.

52. The method of claim 51, wherein the colorimetric detection method uses a lateral flow strip and a colorimetric probe comprising FAM and Biotin.

53. The method of claim 51, wherein the fluorescent detection method uses a fluorescent probe, wherein the fluorescence signal is measured using a fluorometer, a microplate reader, or Real-Time PCR.

54. The method of claim 51, wherein the electrochemical detection method transduces CRISPR-Cas cleavage event to an electrochemical signal using a modified electrode or a nanoparticle-based sensor.
55. The method of claim 48, wherein the sample is a biological, chemical or environmental sample.
56. The method of claim 55, wherein the biological sample is obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, or swab of skin or a mucosal membrane surface or a combination thereof.
57. The method of claim 55, wherein the chemical sample is obtained from a pharmaceutical substance, a chemical reagent, a cosmetic product, an industrial effluent, a pollutant, a hazardous material, a polymer, an adhesive, a solvent, an oil, a gas, a metal alloy, a nanomaterial, a fuel, a cleaning agent, a dye, or a combination thereof.
58. The method of claim 55, wherein the environmental sample is obtained from a food, a beverage, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a freshwater sample, a waste water sample, a saline water sample, exposure to atmospheric air or other gas sample, or a combination thereof.
59. The method of claim 55, wherein the biological, chemical or environmental sample is a crude sample and/or wherein the nucleic acid is not purified or amplified from the sample.
60. The method of any one of claims 48-59, wherein the sample contains a microbe selected from a bacterium, a virus, an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion.
61. The method of claim 60, wherein the virus is selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded

RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus.

62. The method of claim 61, wherein the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mmarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus, Avian paramyxoviruses, penguin or Falkland Islands virus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Betacoronavirus, Betapapillomavirus 1-6, Bhanja virus, Bokeloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepatitis virus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus, Caliciviridae virus, California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chapare mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Fer-de- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarenavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarenavirus, Hantaan virus, Hantavirus Z10, Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus, Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human

papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human
 picomavirus, Human smacovirus, Ikoma lyssavirus, Ilheus virus, Influenza A-C,
 Ippy mammarenavirus, Irkut virus, J-virus, JC polyomavirus, Japanese
 encephalitis virus, Junin mammarenavirus, KI polyomavirus, Kadipiro virus,
 5 Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur
 forest disease virus, Lagos bat virus, Langat virus, Lassa mammarenavirus, Latino
 mammarenavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu
 virus, Louping ill virus, Lujo mammarenavirus, Luna mammarenavirus, Lunk
 virus, Lymphocytic choriomeningitis mammarenavirus, Lyssavirus Ozemoe,
 10 MSS12Y225 virus, Machupo mammarenavirus, Mamastrovirus 1, Manzanilla
 virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle
 virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory
 syndrome coronavirus, Mobala mammarenavirus, Modoc virus, Moijang virus,
 Mokolo virus, Monkeypox virus, Montana myotis leukoencephalitis virus, Mopeia
 15 lassa virus reassortant 29, Mopeia mammarenavirus, Morogoro virus, Mossman
 virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus,
 Nariva virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat
 hepacivirus, Ntaya virus, O'nyong-nyong virus, Oliveros mammarenavirus, Omsk
 hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana
 20 mammarenavirus, Parramatta River virus, Peste-des-petits- ruminants virus,
 Pichande mammarenavirus, Picomaviridae virus, Pirital mammarenavirus,
 Piscihepevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan
 virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta Toro
 virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile
 25 bomavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo
 virus, Rodent Torque Teno virus, Rodent hepacivirus, Ross River virus, Rotavirus
 A-I, Royal Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus,
 Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi
 virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik
 30 virus, Severe acute respiratory syndrome-related coronavirus, Severe fever with
 thrombocytopenia syndrome virus, Shamonda virus, Shimoni bat virus, Shuni
 virus, Simbu virus, Simian torque teno virus, Simian virus 40-41, Sin Nombre

virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taïla virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus, Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus, Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or Zygosaccharomyces bailii virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus, Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof.

63. The method of claim 62, wherein the coronavirus is SARS-CoV-2.

64. The method of claim 60, wherein the bacterium is a Gram-positive bacterium or a Gram-negative bacterium selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile* and *Clostridium tetani*, *Corynebacterium diphtheria*, *Listeria monocytogenes*,

- Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*,
Chlamydia trachomatis, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Neisseria*
meningitidis, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella*
pneumoniae, *Legionella pneumophila*, *Proteus mirabilis*, *Enterobacter cloacae*,
5 *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella*
typhi, and *Vibrio cholera*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*,
Brucella canis, *Brucella ovis*, *Brucella neotomae*, *Mycoplasma mycoides*,
Clostridium perfringens, *Mycobacterium avium* *Salmonella* spp., *Pasteurella*
multocida, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Yersinia*
10 *pestis*, *Francisella tularensis*, *Bacillus anthracis*, *Coxiella burnetii*, *Listeria*
monocytogenes, *Clostridium chauvoei*, *Campylobacter* spp., *Chlamydia psittaci*,
Anaplasma marginale, *Leptospira* spp. Causes leptospirosis, *Bordetella*
bronchiseptica, *Clostridium perfringens*, *Staphylococcus intermedius*, *Bartonella*
henselae, *Chlamydia felis*, *Mycoplasma* spp, *Pasteurella multocida*, *Erwinia*
15 *amylovora*, *Xanthomonas* spp., *Xanthomonas axonopodis*, *Ralstonia*
solanacearum, *Pectobacterium* and *Dickeya* spp., *Agrobacterium tumefaciens*,
Pseudomonas syringae, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas*
campestris pv. *campestris*, *Xanthomonas oryzae*, *Clavibacter michiganensis*,
Xylella fastidiosa, *Pantoea* spp., *Burkholderia glumae*, *Xanthomonas citri*,
20 *Xanthomonas translucens*, *Xanthomonas fragariae*, *Xanthomonas hortorum*,
Clavibacter michiganensis, *Pseudomonas syringae* or a combination thereof.
65. The method of any one of claims 48-64, wherein the method is performed at a temperature of 20-60 °C.
66. The method of claim 65, wherein the method is performed at a temperature of
25 about 37 °C.
67. The method of claim 48, wherein a detection limit of the nucleic acid is at least 0.0015 DNA copies/ μ L.