What is claimed is:

1. A composition when used for detecting activity of an endonuclease, the composition comprising a nucleic acid substrate for detecting activity of an endonuclease comprising:

(a) a donor fluorophore and an acceptor fluorophore, said fluorophores forming a Fluorescence Resonance Energy Transfer (FRET) pair;

(b) at least one structure on at least one nucleic acid strand inhibiting cleavage of the substrate by an exonuclease; and

(c) a recognition sequence and a cleavage site for an endonuclease, wherein the endonuclease is selected from the group consisting of a nucleic acid-guided endonuclease, a zinc finger nuclease (ZFN), a ZFN conjugated to Fok I, a transcription activator-like effector nuclease (TALEN), an Argonaute endonuclease, an Arcus endonuclease, an engineered endoribonuclease; Cas3 and Cas7-11 and a Type II restriction endonuclease;

wherein:

the donor fluorophore and the acceptor fluorophore are not separated by the endonuclease cleavage site; and

the composition further comprises the exonuclease.

2. The composition of claim 1, wherein the exonuclease is selected from Exonuclease I, Exonuclease III, Exonuclease V, Exonuclease VII, Exonuclease VIII, T5 Exonuclease, T7 Exonuclease, Lambda Exonuclease, Exonuclease T, BAL-31 exonuclease, RecJf exonuclease, RNase R, RNase II, RNase D, RNase T, RNase BN, RNase PH, Exoribonuclease I, and Exoribonuclease II.

3. The composition of claim 1 or claim 2, wherein the donor fluorophore is selected from a group consisting of 5-carboxyfluorescein (5-FAM), 6-carboxyfluorescein (6-FAM), 2′,4′,1,4,-tetrachlorofluorescein (TET), 2′,4′,5′,7′, 1,4-hexachlorofluorescein (HEX), 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxyfluorescein (JOE), coumarin dyes, Alexa Fluor dyes, IRDye 800CW, Cascade Blue, Pacific Blue, Pacific Orange, Texas Red, and BODIPY® dyes.

4. The composition of any one of claims 1 to 3, wherein the acceptor fluorophore is selected from a group consisting of tetramethyl-6-carboxyrhodamine (TAMRA), tetrapropano-6-carboxyrhodamine (ROX), DABSYL, DABCYL (4-[[4-(dimethylamino)-phenyl]-azo]-benzoic acid), Cy5 and Cy5.5, anthraquinone dyes, nitrothiazole dyes, nitroimidazole dyes, LC-Red 610, LC-Red 640, LC-Red 705, JA286, DDQ-I, DDQ-II, QSY-7, QSY-21, IRDye QC1, Iowa Black FQ, Iowa Black RQ, HEX (hexachloro-fluorescein), TET (tetrachloro-fluorescein), JOE (5′-Dichloro-dimethoxy-fluorescein), BODIPY® dyes, Eclipse Quencher (4-[[2-chloro-4-nitro-phenyl]-azo]-aniline, BHQ-1 ([(4-(2-nitro-4-methyl-phenyl)-azo)-yl-((2-methoxy-5-methyl-phenyl)-azo)]-aniline), BHQ-2 ([(4-(1-nitro-phenyl)-azo)-yl-((2,5-dimethoxy-phenyl)-azo)]-aniline), and pyridinyl-isoquinoline-dione dyes.

5. The composition of any one of of claims 1 to 4, wherein the exonuclease is selected from Exonuclease III, T5 Exonuclease, T7 Exonuclease, Lambda Exonuclease, and BAL31 Exonuclease, RNase R, RNase II, RNase D, RNase T, RNase BN, RNase PH, Exoribonuclease I, and Exoribonuclease II.

6. The composition of claim 1, wherein the nucleic acid-guided endonuclease is a CRISPR Class I (CASCADE) endonuclease and the substrate comprises a protospacer adjacent motif (PAM) consisting of a sequence selected from 5’-AAG-3’, 5’-AGG-3’, 5’-ATG-3’, 5’-GAG-3’, 5’-CAG-3’, 5’-GTG-3’, 5’-TAA-3’, 5’-TGG-3’, 5’-AAA-3’, 5’-AAC-3’, 5’-AAT-3’, 5’-ATA-3’, 5’-TAG-3’, and 5’-TTG-3’.

7. The composition of claim 1, wherein the nucleic acid-guided endonuclease is a CRISPR Cas9 endonuclease or a CRISPR Cas12a endonuclease and the substrate comprises a protospacer adjacent motif (PAM) consisting of a sequence selected from 5′-NGG-3′, 5′-NGGNG-3′, 5′-NNAAAAW-3′, 5′-NNNNGATT-3′, 5′-GNNNCNNA-3′, 5′-NNNACA-3′, 5’-TTN-3’, 5’-TTTN-3’ and 5’-TTTV-3’.

8. The composition of claim 1, wherein the nucleic acid-guided endonuclease comprises an endonuclease and a nucleic acid targeting nucleic acid (NATNA) and the NATNA comprises DNA and RNA nucleotides.

9. The composition of any one of claims 1 to 8, wherein the structure inhibiting cleavage of the substrate by an exonuclease is selected from a hairpin, a strand overhang, and a nucleic acid modification.

10. A method for detecting activity of an endonuclease in a sample, the method comprising:

(a) contacting the sample with a reaction mixture compising the composition of any one of claims 1 to 9 under conditions suitable for endonuclease and exonuclease cleavage of the nucleic acid substrate; and

(b) measuring fluorescence emitted by the reaction mixture as a result of endonuclease cleavage and subsequent exonuclease cleavage, wherein a change in fluorescence indicates activity of the endonuclease.

11. The method of claim 10, wherein the nucleic acid substrate or the endonuclease are in an unpurified form.

12. A kit when used for detecting activity of an endonuclease comprising the composition of any one of claims 1 to 9.

13. An apparatus when used for detecting activity of an endonuclease by the method of claim 10 or claim 11 comprising a reaction chamber for performing enzymatic reactions and a fluorescence detector.

14. A method for detecting the presence of a target nucleic acid in a sample, the method comprising:

(a) contacting a sample comprising a target nucleic acid that is or has been rendered single-stranded with the composition of any one of claims 1 to 9, wherein the nucleic acid substrate is a probe capable of hybridizing to a target nucleic acid and the probe is or has been rendered single-stranded under conditions suitable for forming a duplex between the probe and the target nucleic acid and for endonuclease and exonuclease cleavage of the substrate; and

(b) measuring fluorescence emitted by the reaction mixture, wherein a change in fluorescence indicates the presence of the target nucleic acid in the sample.

15. The method of claim 14, wherein the sample comprises a crude preparation of nucleic acids.

16. A kit when used for performing a diagnostic procedure consisting of detecting a target nucleic acid according to the method of claim 14, the kit comprising the composition of claim 1, wherein the target nucleic acid is selected from a sequence characteristic of a bacterium, a sequence characteristic of a virus, a sequence characteristic of a parasite, and a patient's sequence characteristic of a patient's disease or condition.

17. A method for optimizing endonuclease digestion reactions, the method comprising:

(a) preparing a series of reaction mixtures with an exonuclease and a nucleic acid substrate according to the composition of any one of claims 1 to 9;

(b) contacting each of the series of reaction mixtures with different amounts of the endonuclease;

(c) measuring fluorescence emitted by the reaction mixture, wherein a change in fluorescence indicates activity of the endonuclease;

(d) selecting the amount of endonuclease yielding the highest fluorescence of the reaction mixture or the highest rate of increase of fluorescence of the reaction mixture as the optimal endonuclease concentration.

18. A method for optimizing CRISPR endonuclease digestion reactions, the method comprising:

(a) preparing a series of reaction mixture with a CRISPR endonuclease, an exonuclease and a nucleic acid substrate comprising:

i. a donor fluorophore and an acceptor fluorophore, said fluorophores forming a Fluorescence Resonance Energy Transfer (FRET) pair;

ii. at least one structure on at least one strand inhibiting cleavage of the substrate by the exonuclease; and

iii. a recognition sequence for an endonuclease;

(b) contacting each of the reaction mixtures in the series with a series of nucleic acid targeting nucleic acids (NATNAs);

(c) measuring fluorescence emitted by the reaction mixture, wherein a change in fluorescence indicates activity of the endonuclease;

(d) selecting the NATNA yielding the highest fluorescence of the reaction mixture as the optimal NATNA.