

Dried Reagents

Field

5 The present invention and disclosure relate to laboratory consumables, and in particular to devices, e.g. multi-well plates, comprising dried reagents for performing detection assay reactions, e.g. immunoassays, to detect target analytes in liquid samples, particularly in multiplex. The device allows a simplified protocol to be performed, and improves assay accuracy.

10 Background

Detection assays for analytes have long been performed to detect a target analyte in a sample, both in clinical settings, and for research, including for the identification of new biomarkers. In particular, the detection of biomarkers, and more generally proteins and other analytes, is of particular interest in the context of liquid samples, especially liquid biopsy samples such as blood. This requires high sensitivity methods with a wide dynamic range, able to detect low abundance analytes in complex samples. Further it is important to be able to perform such detection methods in high multiplex.

Immunoassays using antibody-based target-specific binding probes in various formats have been adopted as the basis for many such analyte detection methods, including proximity assays, such as proximity ligation assay (PLA) and proximity extension assay (PEA). The latter in particular has been developed and commercialized by Olink Proteomics AB, most recently in a next generation sequencing (NGS) readout format (Olink® Explore as described in Wik et al. Mol. Cell. Proteom. Volume 20, 2021, 100168).

Performing immunoassays for multiple samples and multiplex detection of analytes such as proteins requires incubation of samples with a number of reagents. Samples and reagent solutions are added to reaction vessels, e.g. wells in a plate, by manual or automated pipetting. Manual pipetting is laborious and prone to human mistakes. Automated pipetting by lab robots is in general more reproducible but requires investment in obtaining and programming expensive equipment, and tedious processes.

Some immunoassays, such as the Olink® products, use 0.2-1 µL samples. Micro volume liquid handling is challenging to achieve with sufficient precision with most available liquid handlers and with manual procedures.

Many products presently on the market today for multiplexed detection of proteins provide antibody panels in liquid format (e.g. Akoya codex and GeoMx from Nanostring, for example, as well as the Olink® Explore products), with the attendant limitations associated with liquid handling.

5 Providing immunoassay reagents in dried format would be beneficial, both for minimizing liquid handling steps, but also in terms of providing reagents that are stable at room temperature, and easily handled and stored etc. However, despite various proposals for providing dried reagents for immunoassays, such as described in EP0140489, US20080318256, and EP0192320 for example, to the best of our
10 knowledge there is only one product available on the market today which uses dried reagents for immunoassays, for the DURA technique (Beckman), as described in WO 2017/222998. However, this product is only for flow cytometry detection of cell membrane proteins using dye labeled antibodies and with limited plex of assays. It is also not suitable for small volume immunoreactions.

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Summary

The devices and methods herein are directed to providing improved consumables and reagents for conducting proximity-based analyte detection methods, wherein reagents for the detection assay, in particular the analyte-specific
20 proximity probes, which are used to bind to the analyte, are provided in dried form. Proximity assays are based on the principle of dual (or higher) recognition, wherein the proximal binding of at least two analyte-detection probes together to the analyte allows the probes to interact to generate a signal (in other words, at least two probes must bind to the analyte to allow a signal to be generated). In view of this
25 requirement for at least dual binding, proximity assays may be performed using protocols which do not require washing steps (since a single probe will not itself generate a signal). Accordingly, proximity assays may particularly benefit from, and are particularly amenable to, providing the assay reagents in dried format.

Notably, the devices and methods herein are provided for use in detection
30 assay methods using at least one set of analyte-specific proximity probes, wherein the proximity probes in each set each comprise an analyte-binding domain, which as detailed further below, may bind directly or indirectly to an analyte, and a nucleic acid domain. Binding of the proximity probes of a set to an analyte brings the probes into proximity allowing their nucleic acid domains to interact (e.g. by hybridisation to each
35 other or to one or more common oligonucleotides, followed by an extension and/or ligation reaction) and this interaction generates a signal. More particularly, the nucleic acid domains of the proximity probes in a set are used to generate a reporter

nucleic acid molecule which is detected in order to detect the analyte, as is well known in the art, for example, in PLA or PEA reactions.

In this format, the detection assay reagents are pre-dispensed into the wells of a multi-well plate, or other reaction surface/container in whatever is the desired
5 assay device holding or carrying the assay reagents, and are dried. The plate, or more generally device, is thus provided to users in this dried format. The dried reagents are reconstituted into liquid form by addition of a sample, in order to initiate the first of the assay reaction steps (binding of the analyte-specific proximity probes).

Accordingly, in a first aspect, provided herein is a device comprising at least
10 one reaction space with a single pre-dispensed dried reagent composition suitable for performing an assay method for detecting at least one analyte in a sample to be added to the reaction space, wherein the reagent composition comprises at least one analyte-specific proximity probe set and a drying stabiliser, and wherein the proximity probes in each set comprise a binding domain for binding directly or indirectly to the
15 analyte, and a nucleic acid domain.

The stabiliser acts to protect the probes and reagents from damage during the drying process.

Accordingly, the device comprises one or more reaction spaces wherein each reaction space comprises a dried reagent composition disposed thereon or therein
20 (depending on the configuration of the reaction space, the reagent composition may be contained within it, or positioned on it).

Thus, the reaction space may be an area (i.e. a location or position on a surface) or a reaction container. Preferably, the device is for use in multiplex detection assays. This may involve multiple different proximity probes sets being
25 disposed at the same site, or at different sites. Accordingly, in an embodiment, the device comprises multiple separate reaction spaces, e.g. for different samples. In a further embodiment, the device is a multi-well plate, an array or a microfluidic device.

In certain embodiments, the multiple separate reaction spaces are not in liquid communication with one another.

30 In an embodiment, the, or each, reaction space is configured to receive a volume, e.g. a sample volume, of no more than 10 μ l. In other embodiments, larger volumes may be used, e.g. up to 100 μ l.

In particular, the detection assay method generates a reporter nucleic acid molecule, by means of which the analyte is detected. In this regard, the nucleic acid
35 domains of the analyte-specific proximity probes of a set contribute to the generation of, or take part in a reaction which generates, the reporter nucleic acid molecule.

In an embodiment, the analyte-specific proximity probes do not comprise a directly detectable label and/or are not linked to an enzyme.

A set of proximity probes comprises two or more proximity probes, e.g. 2 or 3.

In an embodiment, the reagent composition comprises a pair of analyte-specific proximity probes.

In particular embodiments, the set of analyte-specific proximity probes is a pair of proximity probes for a proximity extension assay (PEA) or proximity ligation assay (PLA).

In certain embodiments for multiplex detection, the reagent composition at each reaction space comprises two or more analyte-specific proximity probe pairs, wherein each probe pair is specific for a different analyte.

In an embodiment, the nucleic acid domain of at least one of the proximity probes in a set of analyte-specific proximity probes comprises an ID sequence, for example a target-specific, or analyte-specific, barcode sequence. In another embodiment, the nucleic acid domains of at least two proximity probes in a set each comprise an ID sequence.

In some embodiments the nucleic acid domain of each proximity probe of a set comprises an ID sequence. In particular, where a proximity probe set comprises two proximity probes (i.e. a proximity probe pair), each of the nucleic acid domains of the pair comprises may comprise an ID sequence.

In any such embodiment, the ID sequences of the proximity probes in a set may be the same or different.

An individual ID sequence may be indicative of an analyte (i.e. analyte-specific). Alternatively a combination of two or more ID sequences may be analyte-specific. Thus, as will be described in more detail below, the reporter nucleic acid molecule which is generated may comprise an analyte-specific combination of ID sequences, which may be viewed, in itself, as an analyte-specific ID sequence derived from the ID sequence(s) of the nucleic acid domains.

In an embodiment, the drying stabiliser comprises one or more sugars or sugar alcohols, e.g. trehalose, glucose, sucrose and/or mannitol, and optionally a surfactant, e.g. a detergent such as Tween.

The reagent composition is typically prepared in a buffer and the buffer composition is dispensed into or onto the reaction space(s) of the device and dried down. The reagent composition thus typically comprises a buffer. The buffer composition that is prepared for drying may contain further reagents for the probe binding step. It may further comprise one or more further reagents for the detection assay method, e.g. for generation of a reporter nucleic acid molecule, for introduction

of a desired sequence element into a reporter molecule (e.g. a barcode or other detection sequence, or a binding site for a primer etc.), for amplification, and/or for detection of a reporter nucleic acid molecule.

5 In an embodiment, the reagent composition further comprises a sample index oligonucleotide comprising an ID sequence, for example for identifying a sample, e.g. an ID sequence which is unique to the reaction space or a group of reaction spaces. The sample ID oligonucleotide is used to incorporate the ID sequence into the reporter nucleic acid molecule. For example, the sample index oligonucleotide is a primer, a ligation adaptor, or a ligation and/or extension template.

10 In an embodiment, the reagent composition further comprises one or more of the following:

- (i) one or more enzymes, e.g. a DNA polymerase and/or a ligase;
- (ii) dNTPs;
- (iii) one or more primers;
- 15 (iv) a ligation template;
- (v) buffers;
- (vi) salts; and/or
- (vii) blocking agents.

20 In a further aspect herein, also provided is a method of performing a detection assay to detect a target analyte in a sample, said method comprising introducing a sample into a reaction space of a device as hereinbefore defined.

A still further aspect provides a method of preparing a device as hereinbefore defined, said method comprising introducing a volume of liquid comprising the reagent composition to the reaction space, and drying said liquid volume to form a
25 dried reagent composition.

More particularly, in this aspect the method comprises preparing a device comprising at least one reaction space with a single pre-dispensed dried reagent composition, said method comprising introducing a volume of liquid comprising the reagent composition as hereinbefore defined to the reaction space, and drying said
30 liquid volume to form a dried reagent composition. In an embodiment, the drying is air-drying, vacuum-drying, freeze drying, or a combination of different drying methods, and if necessary or appropriate multiple drying steps may be used.

A further aspect provides a device obtained or obtainable by the said method.

A yet further aspect provides a kit, or more particularly a kit of parts,
35 comprising a said device and one or more further components selected from:

- (i) an aqueous solution for resuspension, e.g. resuspension of the dried reagents and/or a sample;

(ii) reagents or reaction components for the proximity assay, for example to generate, amplify and/or detect the reporter nucleic acid molecule.

Part (ii) may comprise, or be represented by, a reagent composition comprising the reagents or reaction components.

5 Such reagents or reaction components will generally be reagents or components which are not included (i.e. not incorporated) in the dried reagent composition.

10 In an embodiment, the reagents may be enzymes, notably DNA polymerase and/or DNA ligase. In another embodiment, the reagents may be reagents for performing a nucleic acid amplification reaction. In an embodiment, the amplification reagent composition comprises a DNA polymerase and/or DNA ligase.

Brief Description of Figures

15 Figure 1 shows a flowchart of the standard protocol (A) and the protocol based on dried reagents (B). The standard protocol (A) requires the aliquoting of probes and buffers and mixing in the correct proportion before the adding of samples to start the immunoreaction. The protocol based on dried reagents (B) provides reagents required for the step of incubation with probes of one sample dispensed and dried in each well of the plate, and the sample is added to the dried reagents.
20 The sample is diluted in a 1:3 ratio to compensate for salt present in the dried reagents.

Figure 2 shows the performance comparison between the standard protocol and the protocol based on dried reagents, based on average signal to noise ratio (S/N) (A) and average S/N correlation of all assays (B). (A): Data for the protocol
25 based on dried reagents and RT overnight incubation (New) is represented by the left-sided bar of each column in the chart. 3.5 μ L of probe mixture for drying, 100 mM trehalose and 5mg/ml Tween 80 were used in the protocol based on dried reagents. Data for the standard protocol (Standard) based on wet probes with 4 °C overnight incubation is represented by the right-sided bar of each column in the chart.

30 Figure 3 shows the signal to noise ratio (S/N) (A) and S/N correlation (B) of all assays for sample control. (A): The S/N was calculated by $dCt(Neg)-dCt(Sample)$, wherein dCt is the Ct value normalized with extension control ($Ct(sample)-Ct(Ext Ctrl)$). 3.5 μ L of probe mixture for drying, 100 mM trehalose and 5mg/ml Tween 80 were used.

35 Figure 4 shows the S/N (A) and S/N correlation (B) of each assay in the Olink® Target 48 panel for sample control using either vacuum dried reagents or wet reagents, and with RT overnight incubation for immunoreaction. 10 μ L of probe

mixture for drying and 100 mM trehalose were used in the dried reagent protocol. Tween-80 was not used.

Figure 5 shows the S/N correlation between the protocol based on wet reagents and the protocol based on vacuum dried reagents for each assay in the panel of probes, with NGS as readout. Both protocols used RT over night as the incubation condition. 10 µL of probe mixture for drying and 100 mM trehalose were used in the dried reagent protocol. Tween-80 was not used.

Figure 6 shows the CV% comparison between the protocol based on wet reagents and the protocol based on vacuum dried reagents. The dried reagents generated similar CV% for Neg and PC compared to Wet reagents. PC is the abbreviation for plate control that is used for Olink® Explore panel. Neg is the abbreviation for Negative control for Olink® Explore panel. 10 µL of probe mixture for drying and 100 mM trehalose were used in the dried reagent protocol. Tween-80 was not used.

Detailed Description

The present methods and devices provide improved tools for performing analyte detection assays, and particularly assays which involve the use of analyte-specific proximity probes which comprise a nucleic acid domain which participates in the generation of a reporter nucleic acid molecule by means of which the analyte is detected. Especially, the present methods and devices concern the performance of such assay methods in multiplex, and particularly very high multiplex to detect multiple analytes in a sample, or more particularly in multiple samples. Such methods require high throughput and are typically automated.

As used herein the term “multiple” means two or more in accordance with its usual meaning, or more particularly 3, 4, 5, 6, 10, 15 or 20 or more. However, in the context of the multiplex assays concerned herein, typically much higher numbers are involved, and multiple may be 50, 100, 200, 300, 400, 500 or more.

As used herein, the term “multiplex” is used to refer to an assay in which multiple (i.e. at least two) different analytes and/or multiple different samples are assayed at the same time. More particularly, different analytes may be assayed in the same aliquot of the sample, or in the same reaction mixture, and/or different samples may be analysed in parallel. Thus, the minimum number of assay reactions that may be performed is two. However, it is preferred that considerably more analytes are detected using the present devices and methods. Preferably at least 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400

or 1500 or more analytes are detected. Further, multiple different samples may be handled.

As indicated above, the assays typically require the handling of very small volumes, notably at the microlitre level, and the use of multiple reagents in the various steps of the assay methods. As discussed above, this introduces problems of accuracy, and hence reliability and reproducibility. These problems are addressed herein, by providing at least the initial assay reagents, or key assay reagents, in dried form, providing a much more user-friendly product. The user can simply start the assay by adding sample to the dried reagents, without needing to prepare, mix, or dispense reagents.

Benefits of the present methods and devices thus include:

- analyte-specific proximity probes can be stored together for a long time without producing background.
- sample index oligonucleotides can be present initially and not added at a separate step.
- reduced need for accurate liquid handling, especially small volume accurate pipetting, which is especially problematic for sticky and high viscosity samples.
- reduction of steps in complex protocols, especially reduced need for automated liquid handling, resulting in reduced need for investments in expensive equipment.
- No need for cold chain shipment of reagents. Reduces costs and environmental impact.

The need for human intervention is reduced, thus reducing the risk of errors, contamination and imprecision. In running an automated system, the need for human hands-on time is reduced. The improvements herein, namely the provision of assay reagents in dried format, allow the performance of complex methods of analyte detection which rely on the generation of a nucleic acid reporter for the analyte, to be simplified and improved.

As indicated above, the preparation of the devices herein involves preparing a reagent composition in liquid form, typically in aqueous liquid form, dispensing the composition into one or more reaction spaces comprised in or on the device, and drying the reagent composition to form a dried reagent composition in or on the reaction space.

As noted above the user can simply add the sample to be tested to the reaction space. The sample is added in liquid form, and reconstitutes the reagent

composition into liquid form. The device (or in other words, the sample and reagent composition mixture) is incubated to allow the analyte detection proximity assay to be initiated, or for the analyte detection proximity assay to be performed. Notably, this allows the analyte-specific proximity probes in the reagent composition to bind to their target analyte (i.e. the analyte to be detected by that set of proximity probes). This will be described further below. The sample may be used directly, or it may be diluted, or prepared in any convenient way, to provide a sample for use with the device.

The device can be any device suitable for, and known in the art for, performing analyte detection assays. Thus, it may be viewed, or referred to, as a reaction vessel. The device has one or more reaction spaces where an analyte detection assay reaction may be performed, but typically it will have multiple such spaces, for performing multiple assay reactions in parallel, for example on different samples, or on different aliquots for portions of the same sample, e.g. at different dilution. One or more reaction spaces may also be used for performing control reactions. The device can have various different formats and configurations, depending e.g. on the assay reaction or detection technology, or the instrumentation used etc. Generally, however, it will have a planar configuration. Representative device formats include plates, typically multi-well plates, e.g. micro-titre plates, slides, e.g., with compartments, tubes, arrays, and microfluidic circuits. Any device format in or on which one or more reaction spaces may be provided is included. The device is thus a body which can house or carry one or more reaction space(s).

A reaction space is a location where a reaction can take place. In other words, a reaction space is a location where the reagent composition, when reconstituted by addition of sample, can be confined to allow the reagent composition to react, or interact, with the sample. The configuration of the reaction space can depend, inter alia, on the volume of sample intended to be used. The reaction space may be seen as a surface or substrate where the reaction can take place. The reaction space, and indeed the device, can be composed of any suitable material, including but not limited to plastic, nitrocellulose, cellulose acetate or glass. The reaction space can be formed as part of the inside surface of the body of the device, e.g. a tube, or a well of a plate, or a conduit in a flow circuit etc. The reaction space can thus be a compartment in or on a device.

Whilst in many typical devices, the reaction space is a discrete physical compartment, i.e. a reaction container, which may be defined by a physical structure (e.g. walls, or bottom surface etc.) which delimits the reaction space and contains the reaction mixture resulting from contact with the sample, the present devices are not

limited to this, and include any area where a reaction can take place. Thus, the reaction space can simply be an area of surface, i.e. a position or location, where the dried composition is deposited. This includes, for example, a spot on an array etc.

In the devices herein, where there are multiple reaction spaces, they are in particular embodiments discrete. That is, the multiple reaction spaces are configured to allow multiple separate assay reactions to take place, each at an individual reaction space. In other words, in such an embodiment, the multiple reaction spaces are not in liquid communication with one another – once reconstituted by addition of sample (i.e. following the addition of the sample), the resulting reaction mixture at one reaction space stays physically separate from the reaction mixture at another reaction space. Thus, in such embodiments, it is not included that the reagent compositions from different reaction spaces become mixed, or come into contact with another (at least not until after the assay detection reactions have been initiated, or at least until the assay specific proximity probes have become bound to their target analytes, or more particularly, until after reporter nucleic acids have been generated in the individual assay reactions). In particular, the reagent mixture from one reaction space cannot simply flow to another reaction space, unless a physical transfer step is performed by the user or instrument to move the reaction mixture to another location. In other words, a single reaction mixture is not formed or reconstituted from different reaction spaces, or from different dried reaction compositions, for the initial detection assay reaction. Accordingly, in an embodiment, each reaction space is configured to receive a liquid sample which individually reconstitutes (e.g. dissolves and/or suspends) the reagent composition in that space.

Each reaction space comprises a single dried reaction composition. Thus, the reaction mixture which is formed at each reaction space from contact with the sample is formed from a single dried composition, and is not constituted from more than one dried composition. It is not included that multiple dried compositions are reconstituted together to form a single reaction mixture. As will be described in more detail below, the dried reagent composition is a mixture of reagents comprising at least one set of analyte-specific proximity probes and a drying stabiliser. Addition of sample to the reaction space, allows the analyte-specific proximity probes to bind to the target analyte without the addition of further reagents (although these may be added for downstream reactions, following analyte-binding).

The analyte which is to be detected using the devices and methods herein (referred to as the “target analyte”) may be any substance (e.g. molecule) it is desired to detect.

The analyte may accordingly be any biomolecule or chemical compound it is desired to detect, for example a peptide or protein, or a nucleic acid molecule or a small molecule, including organic and inorganic molecules. The analyte may be a cell or a microorganism, including a virus, or a fragment or product thereof. It will be seen
5 therefore that the analyte can be any substance or entity for which a specific binding partner (e.g. an affinity binding partner) can be developed. All that is required is that the analyte is capable of being bound by an analyte-specific probe comprising a binding domain (i.e. a binding partner) for the analyte.

Proximity assays, for which the present devices and methods may be used,
10 have particular utility in the detection of proteins or polypeptides. Analytes of particular interest thus include proteinaceous molecules such as peptides, polypeptides, proteins or prions or any molecule which includes a protein or polypeptide component, etc., or fragments thereof. In a particularly preferred embodiment, the analyte is a wholly or partially proteinaceous molecule, most
15 particularly a protein. That is to say, it is preferred that the analyte is or comprises a protein (the term "protein" is used broadly herein to include peptides or polypeptides).

The analyte may be a single molecule or a complex that contains two or more molecular subunits, which may or may not be covalently bound to one another, and which may be the same or different. Thus, in addition to cells or microorganisms,
20 such a complex analyte may also be a protein complex, or a biomolecular complex comprising a protein and one or more other types of biomolecule. Such a complex may thus be a homo- or hetero-multimer. Aggregates of molecules e.g. proteins may also be target analytes, for example aggregates of the same protein or different proteins. The analyte may also be a complex between proteins or peptides and
25 nucleic acid molecules such as DNA or RNA. Of particular interest may be the interactions between proteins and nucleic acids, e.g. regulatory factors, such as transcription factors, and DNA or RNA. Thus, in a particular embodiment the analyte is a protein-nucleic acid complex (e.g. a protein-DNA complex or a protein-RNA complex). Also included is the detection of protein modifications, including post-
30 translational modifications (PTMs). Thus, for example, a protein may be detected, and it may also be detected whether a modifying group, e.g. a phosphate group, is present in the protein. In another embodiment, the analyte is a non-nucleic acid analyte, by which is meant an analyte which does not comprise a nucleic acid molecule. Non-nucleic acid analytes include proteins and protein complexes, as
35 mentioned above, small molecules and lipids.

Where multiple analytes are to be detected, they may be of the same type (e.g. all the analytes may be proteins, or protein complexes), or of different types

(e.g. some analytes may be proteins, others protein complexes, others lipids, others protein-DNA or protein-RNA complexes, etc., or any combination of such types of analytes).

The term "detecting" or "detected" is used broadly herein to include any
5 means of determining the presence or absence of an analyte (i.e. determining whether a target analyte is present in a sample of interest or not). Detecting an analyte may include any form of measurement of the concentration or abundance of the analyte in the sample. Either the absolute concentration of a target analyte may be determined, or a relative concentration of the analyte, for which purpose the
10 concentration of the target analyte may be compared to the concentration of another target analyte (or other target analytes) in the sample or in other samples.

Thus "detecting" may include determining, measuring, assessing or assaying the presence or absence or amount of an analyte in any way. Quantitative and qualitative determinations, measurements or assessments are included, including
15 semi-quantitative determinations. Such determinations, measurements or assessments may be relative, for example, when two or more different analytes in a sample are being detected, or absolute. As such, the term "quantifying" when used in the context of quantifying a target analyte in a sample can refer to absolute or to relative quantification. Absolute quantification may be accomplished by inclusion of
20 known concentration(s) of one or more control analytes and/or referencing the detected level of the target analyte with known control analytes (e.g. through generation of a standard curve) (described in more detail below). Alternatively, relative quantification can be accomplished by comparison of detected levels or amounts between two or more different target analytes to provide a relative
25 quantification of each of the two or more different analytes, i.e. relative to each other.

The sample may be any sample of interest – that is to say, any sample which contains or may contain analytes of interest. It may be any biological or clinical sample, e.g. any cell or tissue sample of or from an organism, or any body fluid or preparation derived therefrom, as well as samples such as cell cultures, cell
30 preparations, cell lysates etc. Environmental samples, e.g. soil and water samples, or food samples are also included. The samples may be freshly prepared or they may be prior-treated in any convenient way e.g. for storage.

The sample is a liquid sample. Such a sample typically comprises target analytes obtained as, or dispersed in, a predominantly aqueous medium. Thus, the
35 sample may be obtained as a liquid sample, or prepared as a liquid sample. The sample may be resuspended in an aqueous solution and the resuspended sample introduced into the reaction space. Alternatively, an aqueous solution may be

introduced into the reaction space and the sample resuspended in the aqueous solution after introduction into the reaction space. The aqueous solution may comprise a surfactant.

Representative samples thus include any material which may contain a
5 biomolecule, or any other desired or target analyte, including for example foods and allied products, and clinical and environmental samples. The sample may be a biological sample, which may contain any viral or cellular material, including prokaryotic or eukaryotic cells, viruses, bacteriophages, mycoplasmas, protoplasts and organelles. Such biological material may thus comprise any type of mammalian
10 and/or non-mammalian animal cell, plant cells, algae including blue-green algae, fungi, bacteria, protozoa etc.

In an embodiment, the sample is a clinical sample, for instance whole blood and blood-derived products such as plasma, serum, buffy coat and blood cells, urine, faeces, cerebrospinal fluid or any other body fluid (e.g. respiratory secretions, saliva,
15 milk etc.), and biopsies. It may thus be a liquid biopsy sample or a tissue biopsy sample which is prepared as a liquid sample. Plasma or serum samples are of particular interest. The sample may in particular be derived from a human, though it may equally be derived from non-human animals (i.e. veterinary samples). The sample may be pre-treated in any convenient or desired way to prepare it for use, for
20 example by cell lysis or removal, etc.

The sample may contain multiple analytes, including which analytes have varying levels of abundance in the sample. That is to say, the analytes are present in the sample at different concentrations, or at a range of concentrations.

As mentioned above, the sample may be used as is, e.g. a plasma or serum
25 sample as obtained from a subject, or it may be prepared, e.g. resuspended. In particular, it may be diluted. It may be convenient to dilute the sample for use in the device. This may be to account for different abundances of analytes in a sample, e.g. to increase the dynamic range of the detection assay, so as to be able to detect both high and low abundance analytes in the same sample, and/or in view of the nature of
30 the sample, for example for sticky or hard-to-handle samples. A method for handling samples comprising multiple analytes present in different amounts is described in WO 2021/191442, in which analytes are "blocked" or grouped, and analysed in different portions or aliquots of a sample, and the devices and methods herein are particularly suitable for use in the methods described therein (WO 2021/191442 and
35 other documents referenced herein are incorporated by reference).

The sample may be diluted in any suitable medium, and at any suitable dilution factor according to methods and procedures known in the art. For example,

the sample may be diluted at a factor of X2 to X10, e.g. 2- to 8-, 6-, 5- or 4-fold etc. If necessary, higher dilution factors may be used. In some embodiments, a dilution series of the sample may be employed, with different dilutions being assayed in different reaction spaces. For instance, samples may be diluted 1:2, 1:3, 1:4, 1:5, 5 1:10, etc. In particular, aliquots may be subjected to 10-fold dilutions, i.e. one or more aliquots may be diluted 10-fold (or 1:10), one or more aliquots may be diluted 100-fold (1:100), and one or more aliquots may be diluted 1000-fold (1:1000) or more. One or more aliquots may be undiluted (referred to herein as 1:1).

Dilutions of the sample may be made with any suitable diluent, which may 10 depend on the type of sample being assayed. For instance, the diluent may be water or saline solution, or a buffer solution, in particular a buffer solution comprising a biologically-compatible buffer compound (i.e. a buffer compatible with the detection assay used, for instance a buffer compatible with a PEA or PLA). Examples of suitable buffer compounds include HEPES, Tris (i.e. 15 Tris(hydroxymethyl)aminomethane), disodium phosphate, etc. Suitable buffers for use as a diluent include PBS (phosphate-buffered saline), TBS (Tris-buffered saline), HBS (HEPES-buffered saline), etc. The buffer (or other diluent) used ideally is made up in a purified solvent (e.g. water) such that it does not contain contaminant analytes. The diluent should thus be sterile, and if water is used as a diluent or the 20 base of the diluent, the water used is preferably ultrapure (e.g. Milli-Q water).

The reaction space(s) of the device are configured to receive a desired volume of sample (whether diluted or not, in the form in which it is to be used in the detection method). Advantageously, the device can be designed to receive small, or very small reaction volumes, particularly at the micro level, or even at the nano level. 25 In an embodiment, the reaction space is configured to receive a sample volume of no more than 20 or 15 μl , particularly not more than 10 μl . In some embodiments, this may be no more than 5, 4, 3, 2, or 1 μl , e.g. 0.2 to 1 μl . This may be suitable for e.g. microtitre plates. In other embodiments, even smaller volumes may be used, for example in the context of arrays and such like, for example up to 100 nL. Alternatively 30 in still other embodiments, larger volumes may be used, for example up to 500, 400, 300, 200 or 100 μl .

The reaction spaces are also designed to receive a volume of liquid reagent composition to be dried, in such a manner that a defined reaction space with the dried reagent composition is created. This volume may be larger than the sample 35 volume. The drying volume may for example be 3-10 μl . The drying volume will depend on the configuration of the device and reaction space(s) and also can be varied according to choice, for example depending on the assay reagents, stabilisers

etc., and desired dilutions for preparing reagents etc. In the examples below, drying volumes up to 10 µl were evaluated, but these could be larger, e.g. up to 15, 20, 25, 30, 40, 50 or 100 µl, or even higher, as discussed above for sample volumes.

Likewise, in accordance with the smaller, nanolitre level, sample volumes above, the
5 reaction spaces may be designed to receive smaller drying volumes, for example in a microarray format.

The reagent composition may be prepared in liquid form and dispensed, or delivered, to the reaction space(s) in any convenient way, e.g. manually or by instrument.

10 Drying may be by any convenient process, and includes air-drying, vacuum-drying or freeze-drying. Drying means simply that the reagent composition is dried, i.e. liquid (e.g. water) is removed until the composition is dried (substantially free of liquid/water). Air- and vacuum-drying are convenient, and freeze-drying is not necessary to preserve the reagents. In an embodiment, the reagent composition is
15 not freeze-dried (lyophilised). However, in other embodiments this is not excluded.

Procedures and conditions for drying are known in the art, and may vary depending on the reagents, e.g. on the analyte-specific proximity probes which are used. Thus, suitable temperatures and atmospheric pressures for the drying process may be varied, and selected, according to choice.

20 For example, vacuum drying may take place in a vacuum chamber for 1 to 6 hours, e.g. 2 hours, at a temperature of 20 to 40 °C, e.g. 30 to 35 °C, for example at 35 °C. Time and temperature may be varied depending on drying volume and/or nature and concentration of drying stabiliser etc. The skilled person may readily determine this according to routine skill and procedure. Similarly, conditions for air-
25 drying, e.g. oven-drying, may readily be determined, for example, time, air flow and temperature in an oven. In another embodiment, the reagent composition can be dried at room temperature.

The liquid reagent composition which is dried comprises one or more drying stabilisers. Such stabilising agents, which protect the reagents in the composition
30 during the drying process, are known in the art, and any known or available drying stabilisers may be used, singly or in any combination. Such drying stabilisers include proteins, carbohydrates or organic polymers. Suitable proteins include inert proteins such as are routinely used as blocking agents, e.g. to block non-specific binding of proteins to surfaces, and included for example, albumin proteins, such as BSA,
35 gelatin, or milk proteins (e.g. cases, or dried milk powder). Carbohydrates include particularly sugars, e.g. glucose or glucose-containing sugars such as trehalose,

dextrose or sucrose, or sugar alcohols, e.g. mannitol or sorbitol etc. The polymer may for example be polyvinyl pyrrolidone or polyethylene glycol.

Suitable or optimum concentrations for the drying stabiliser may be selected according to protocols known in the art, or may be determined empirically for a given reagent composition by routine experiments. For example, a sugar such as trehalose, for example, may be used at a concentration of 10 to 500 mM, or 20-400 mM, e.g. 50-300 mM (final concentration in the liquid reagent composition). In the Examples below, 100 mM trehalose was found to be suitable. Other sugars, e.g. glucose and sucrose, were evaluated with similar results.

The drying stabiliser may optionally comprise additional components, for example, surfactants, e.g. detergents. Suitable materials for such use are known in the art, and include, for example, non-ionic detergents, such as those from the Tween, Brij, or Triton groups. Typical detergents suitable for this use are based on a polyoxyethylene or glycoside. In an embodiment the surfactant is a Tween detergent. Tween detergents contain a hydrophilic ethylene glycol head group and a hydrophobic alkyl tail, which may vary in length. Examples include Tween 20, Tween 40 and Tween 80.

The precise nature, or composition of the drying stabilisers used may be selected according to choice and the optimum components and concentrations for each reagent composition may be determined by routine tests. Further, the drying stabiliser composition may vary for different assay formats and probe types, and indeed certain stabilisers may be more beneficial for certain specific analyte-specific probes than for others. Thus, the stabiliser composition may be varied depending on the particular probes and assay reagents used etc.

A representative drying stabiliser composition comprises trehalose. Another representative stabiliser composition comprises trehalose and Tween 80.

As noted above, suitable concentrations for the detergent may be determined empirically by routine tests. However, for Tween detergents, e.g. Tween 80 concentrations of 5-10 mg/ml have been found to be suitable. Thus, a concentration range for a surfactant or detergent may be 3-15, e.g. 5-15, 5-12 or 5-10 mg/ml.

After drying, the devices may be packaged and sealed to protect them for storage. Materials and procedures for this are known in the art. For example, the device may be packaged in a metal membrane which is heat sealed. A second packaging layer may be used, for example, a vacuum sealing bag, which may include a desiccant material, e.g. a silica gel bag.

The reagent composition comprises at least the analyte-specific proximity probes, which are used to bind to the target analyte in the first step of the analyte-

detection method. Depending on the detection assay, this may be two or more analyte-specific proximity probes for each analyte, as commonly used on proximity assays (where 2 or more probes are provided for each target analyte, e.g. a proximity probe pair, where both (or all if more than 2) are capable of binding simultaneously to the target analyte, in proximity to each other.

The analyte-specific proximity probes are typically provided in a composition in which they may be used, i.e. which provides suitable conditions to allow the probes to bind, and to interact with each other.

Typically, the reagent composition comprises a buffer. Analyte-specific proximity probes are routinely provided in the art in a so-called incubation buffer, and any incubation buffer known or available in the art may be used to prepare the liquid reagent composition which is dried. Thus, the dried reagent composition may comprise, in addition to the analyte-specific proximity probe(s) and drying stabiliser, a buffer, or more particularly a buffer composition. This may, for example, additionally comprise one or more salts, and optionally one or more other components, for example, selected from chelators, and surfactants. Other optional components include blocking agents, e.g. non-specific blocking agents such as proteins e.g. BSA, as well as blocking agents designed or provided for use with the detection method. The liquid composition which is dried, advantageously in one embodiment, may have physiological salt concentration and/or physiological pH.

By way purely of representative example, an exemplary incubation solution which may be used to prepare a liquid reagent composition suitable for use with the Explore PEA of Olink Proteomics AB, as described in the Examples below, is set out in Table 1.

Table1 – Incubation Solution

Component	Concentration
Triton X-100	1.70 g/l
NaCl	8.01 g/l
KCl	0.2 g/l
Na ₂ HPO ₄	1.44 g/l
KH ₂ PO ₄	0.2 g/l
EDTA Na-salt	1.24 g/l
BSA	8.80 g/l
Blocking-probes Mix	0.199 g/l
GFP	1-5 pM

Similar ingredients and concentrations may be selected and prepared, based on those used in Table 1, to prepare reagent compositions for other detection assays.

5 The liquid reagent composition which is dried may comprise one or more other reagents which are used for performance of the assay, or for detection of the analyte. This may include reagents for the generation of a reporter nucleic acid molecule, for amplification (e.g. of the reporter nucleic acid molecule), and/or for the detection of a reporter nucleic acid molecule. These will, of course, depend on the nature of the detection assay, as described in more detail below. In one embodiment, 10 the reagent composition comprises all the assay reagents required for the detection assay, but this is not an absolute requirement. Accordingly, included herein are embodiments, where not all the assay reagents are included, and one or more assay reagents are added to, or are contacted with the device (or the reaction spaces thereof) in use.

15 As noted above, and described further below, an analyte-specific proximity assay is based on the binding of two or more proximity probes to create a signal. In this regard, an analyte-specific assay for a given analyte uses a set of proximity probes. The reagent compositions herein comprise one or more sets, each set specific to an analyte. Each set may comprise two or more proximity probes.

20 Proximity probes are typically present in analyte-specific pairs, however they may also exist in larger analyte-specific sets, for example groups of 3 or 4.

Proximity probes within a set create a signal when they are each bound to the target analyte. Binding of the probes to the analyte brings them into proximity, and this proximity allows their nucleic acid domains to interact. Thus signal generation is 25 dependent on an interaction between the probes (more particularly between their nucleic acid domains) and hence typically only occurs when the necessary probes have bound to the analyte, thereby lending improved specificity to the detection system. As noted above, the interaction typically involves the nucleic acid domains binding (hybridising) to one another, e.g. as in a PEA, or to another nucleic acid 30 molecule, e.g. a ligation template or so-called "splint oligonucleotide" (e.g. as in a PLA), or the nucleic acid domains may together template the ligation and/or extension of added oligonucleotides, and in certain embodiments a nucleic acid domain might prime an extension or amplification reaction (e.g. amplification of a ligation product).

35 The interaction of the nucleic acid domains generates a reporter nucleic acid molecule, and this may be achieved in a variety of ways. This may include amplification, extension, ligation and/or cleavage.

In PEA, typically nucleic acid domains linked to the analyte-binding domains of a probe pair hybridise to one another when the probes are in close proximity (i.e. when bound to a target), and are then extended using a nucleic acid polymerase. The extension forms a reporter nucleic acid (the extension product), detection of
5 which demonstrates the presence in a sample of interest of a particular analyte (the analyte bound by the relevant probe pair). In PLA, nucleic acid domains linked to the analyte-binding domains of a probe pair come into proximity when the probes of the probe pair bind their target, and may be ligated together, or alternatively they may together template the ligation of separately added oligonucleotides which are able to
10 hybridise to the nucleic acid domains when they are in proximity. The ligation product is then amplified, acting as a reporter nucleic acid.

The analyte-specific proximity probes comprise a binding domain coupled to a nucleic acid domain. The binding domain may bind to the target analyte directly or indirectly. Thus, the probes may be primary reagents which bind to the analyte
15 directly. Alternatively, they may be secondary reagents which bind to the target analyte indirectly, i.e. they bind to a primary, or intermediary, binding partner which is itself directly bound to the analyte. In the case of the latter, both the primary and secondary partners are provided in the dried reagent composition.

The binding domain may comprise any binding partner (i.e. any entity)
20 capable of binding specifically to the target analyte or a part thereof, or to a primary binding partner therefor. In a particular embodiment, the binding domain is specific for an analyte, i.e. it is an analyte-binding domain. By "specific for an analyte" it is meant that the binding domain specifically recognises and binds a particular target analyte, i.e. it binds its target analyte with higher affinity than it binds to other
25 analytes or moieties.

As is known to the skilled person, that a binding domain is "specific" to or for a certain analyte means it recognizes the analyte with low cross-reactivity (off-target binding) with other potentially present analytes, within the relevant application and experimental context. A framework for determining specificity for binders has been
30 established by an International Working Group for Antibody Validation (Uhlén *et al.* Nat Methods, 2016 Oct; 13(10), 823-827, incorporated herein by reference).

Typically, the analyte-specific binding domain may be a protein, for example, an antibody, or an antigen-binding part thereof, including, but not limited to, monoclonal, recombinant monoclonal, and polyclonal antibodies and antigen-binding
35 antibody derivatives and fragments.

In certain embodiments, the binding domain is preferably an antibody, in particular a monoclonal antibody, or an antibody fragment or derivative thereof comprising the antigen-binding domain. Examples of such antibody fragments or derivatives include Fab, Fab', F(ab')₂, Fv fragments, diabodies, single-domain antibodies (sdAb, Desmyter et al. (1996) Nat. Structure Biol. 3:803-811, incorporated herein by reference), nanobodies, single-chain Fv (scFv, Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85, 5879-5883, incorporated herein by reference), divalent scFV (di-scFvs), tandem scFvs, triabodies, diabodies, single-chain diabodies (scDb), bi-specific T-cell engagers (BiTEs, Kufer et al. (2004) Trends Biotechnol. 22:238-244, incorporated herein by reference), and Dual Affinity Retargeting molecules (DARTs, diabodies additionally stabilized through a C-terminal disulfide bridge). The specificity of analyte-specific reagents with regard to the intended detection assay may be evaluated using the framework proposed by the International Working Group for Antibody Validation, cited above.

However, the analyte binding domain may be of any nature, including lectins, soluble cell surface receptors, receptor ligands, combinatorially derived proteins from phage display or ribosome display, peptides, carbohydrates or molecularly imprinted polymers (MIPs).

Other binding partners may also be used, including for example nucleic acids, which may bind by hybridisation (i.e. a nucleic acid molecule comprising the complementary sequence for a target nucleic acid), or they may be aptamers. Other binding partners may include enzymes or substrates or inhibitors or co-factors (depending on which is the target analyte), carbohydrates, biotin and avidin or streptavidin or such like.

Binding domains may be used in in any combination.

Reagents useful as analyte binding domains are commercially available from a number of manufacturers that offer off-the-shelf reagents or develop new binding reagents for specific analytes and specific needs. Such manufacturers include, among others, Thermo Fisher Scientific (Boston, MA, USA), Abcam (Cambridge, United Kingdom), Bio-Techne (Minneapolis, MN, USA), Proteogenix (Schiltigheim, France), Sino Biological (Beijing, China), Agrisera (Vännäs, Sweden), Novaptech (Pessac, France), Aptamer Group (York, United Kingdom). Reagents useful as analyte binding domains may also be developed independently of commercial suppliers, according to protocols well-known to the skilled person. Such protocols are e.g. described in "Monoclonal Antibody Production" (National Academy Press, Washington, DC, USA, 1999), Carey-Hanly et al. (ILAR Journal, Volume 37, Issue 3,

1995, Pages 93–118), Ilgu and Nilsen-Hamilton (Analyst. 2016 March 7; 141(5): 1551–1568), which are incorporated herein by reference.

In addition to the analyte-binding domain, a proximity probe also comprises a nucleic acid domain, which may also be referred to as an oligonucleotide. The
5 oligonucleotide must be long enough to comprise the necessary functional elements used in the proximity assay. In particular, it is long enough to comprise, at least a sequence capable of generating an identification (ID) sequence in the reporter molecule. This is typically 5-20 nucleotides, such as 5-10, 5-15, 10-15 or 15-20 nucleotides. The oligonucleotide may also contain sequences related to primer sites
10 and/or sequencing adaptors for read-out, as known in the art. Generally, the oligonucleotide has a length in the range of 20-100 nucleotides, but may be shorter or longer as required in the specific proximity assay in which the proximity probe is intended to be used.

Conjugation of a nucleic acid moiety to an analyte-binding domain (e.g.
15 antibody) can be performed in several ways known to the skilled person, e.g. as reviewed by Dugal-Tessier et al. (J.Clin.Med.2021,10,838, incorporated herein by reference). Commercial kits for preparing antibody-oligonucleotide conjugates are also readily available from a number of suppliers. The oligonucleotides may be coupled to the analyte-binding domains by any means known in the art, and which
20 may be desired or convenient and may be direct, or indirect, e.g. via a linking group. For example, the domains may be associated with one another by covalent linkage (e.g. chemical cross-linking) or by non-covalent association e.g. via streptavidin-biotin based coupling (biotin being provided on one domain, particularly the oligonucleotide domain, and streptavidin on the other).

25 The oligonucleotide and analyte-binding domain are joined together either directly through a bond or indirectly through a linking group. Where linking groups are employed, such groups may be chosen to provide for covalent attachment of the nucleic acid moiety and analyte-binding domain through the linking group. The linking group, when present, is in many embodiments biologically inert. In representative
30 embodiments, the linking group is generally at least about 50 Daltons, usually at least about 100 Daltons and may be as large as 1000 Daltons or larger, for example, up to 1000000 Daltons if the linking group contains a spacer, but generally will not exceed about 500 Daltons and usually will not exceed about 300 Daltons. Generally, such linkers will comprise a spacer group terminated at either end with a reactive
35 functionality capable of covalently bonding to the nucleic acid domain or analyte binding domain. Spacer groups of interest may include aliphatic and unsaturated hydrocarbon chains, spacers containing heteroatoms such as oxygen (ethers such

as polyethylene glycol) or nitrogen (polyamines), peptides, carbohydrates, cyclic or acyclic systems that may possibly contain heteroatoms. Spacer groups may also be comprised of ligands that bind to metals such that the presence of a metal ion coordinates two or more ligands to form a complex. Specific spacer elements include: 1,4-diaminohexane, xylylenediamine, terephthalic acid, 3,6-dioxaoctanedioic acid, ethylenediamine-N,N-diacetic acid, 1,1'-ethylenebis(5-oxo-3-pyrrolidinecarboxylic acid), 4,4'-ethylenedipiperidine.

Potential reactive functionalities include nucleophilic functional groups (amines, alcohols, thiols, hydrazides), electrophilic functional groups (aldehydes, esters, vinyl ketones, epoxides, isocyanates, maleimides), functional groups capable of cycloaddition reactions, forming disulfide bonds, or binding to metals. Specific examples include primary and secondary amines, hydroxamic acids, N-hydroxysuccinimidyl esters, N-hydroxysuccinimidyl carbonates, oxycarbonylimidazoles, nitrophenylesters, trifluoroethyl esters, glycidyl ethers, vinylsulfones, and maleimides.

Specific linker groups that may find use in the subject proximity probes include heterofunctional compounds, such as azidobenzoyl hydrazide, N-[4-(p-azidosalicylamino)butyl]-3'-[2'-pyridyldithio]propionamide, bis-sulfosuccinimidyl suberate, dimethyladipimidate, disuccinimidyltartrate, N-maleimidobutyryloxysuccinimide ester, N-hydroxy sulfosuccinimidyl-4-azidobenzoate, N-succinimidyl [4-azidophenyl]-1,3'-dithiopropionate, N-succinimidyl [4-iodoacetyl]aminobenzoate, glutaraldehyde, and succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate, 3-(2-pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP), 4-(Nmaleimidomethyl)-cyclohexane-1-carboxylic acid N-hydroxysuccinimide ester (SMCC), and the like.

In one embodiment, the proximity probes are manufactured by coupling a universal oligonucleotide to the analyte-binding domain and subsequently hybridizing a tag oligonucleotide to the universal oligonucleotide, wherein the tag oligonucleotide comprises a sequence capable of generating the identification (ID) sequence in a reporter molecule, a sequence complementary to the universal oligonucleotide to facilitate hybridization, and any other functional sequences necessary to perform the proximity assay for which the proximity probes are intended. Methods for manufacturing such proximity probes are described *inter alia* in international patent publication WO2017/068116, incorporated herein by reference.

The nucleic acid domain may be DNA or RNA, or any combination or modification thereof, e.g. PNA or other derivatives containing non-nucleotide

backbones, or indeed any suitable nucleic acid that is capable of Watson-Crick base pairing. Thus, the nucleic acid domain of the proximity probes may be made up of ribonucleotides and/or deoxyribonucleotides as well as synthetic nucleotide residues that are capable of participating in Watson-Crick type or analogous base pair

5 interactions. Generally speaking the nucleic acid domain is capable of participating in a nucleic acid ligation and/or extension reaction, e.g. is capable of serving as an extension template and/or of being extended, or is capable of being ligated. In particular, it is a DNA domain. Depending on assay design, the nucleic acid domain may be single- or double-stranded, in particular partially double-stranded, comprising

10 both a single-stranded part and a double-stranded part.

In an embodiment, the analyte-specific proximity probe does not comprise a directly or indirectly detectable label, e.g. a fluorescent or coloured label or a mass label or particulate label, or a colorimetric label, e.g. an enzyme substrate, or is not linked to an enzyme, notably an enzyme which is used in the detection reaction, e.g.

15 to generate a detectable product, such as in an ELISA or similar for example. In a more particular embodiment, the analyte-specific probe does not comprise a directly-detectable label and/or is not linked to an enzyme.

In another embodiment the analyte-specific proximity probes are not immobilised to the reaction space. In particular, this means that the analyte-specific

20 proximity probe is not bound to the reaction space, such that it can be reconstituted in solution or suspension as part of a liquid reaction mixture, e.g. to form a homogenous reaction mixture. In other words, in the dried composition the analyte-specific proximity probe is only attached to the reaction space by means of being deposited by drying and not in any other way.

25 As noted above, the nucleic acid domains of the proximity probes are used in the assay method to generate a nucleic acid reporter molecule which is detected in order to detect the analyte.

The detection method is a proximity assay, and the set of analyte-specific proximity probes or the assay of a given analyte is, or comprises, at least a pair of

30 analyte-specific proximity probes. Thus, in an embodiment, the reagent composition comprises a pair of analyte-specific proximity probes (in the sense that the probes are used in an analyte-specific manner, to detect a specific target analyte). In more particular embodiments, the proximity probes are PEA or PLA probes. A proximity probe pair can be defined as comprising forward and reverse probes, or matched or

35 paired probes (as indeed a proximity probe set can be defined as comprising matched probes).

Proximity assays and proximity probes comprising nucleic acid domains are widely known and described in the art. When the proximity probes have bound in proximity to their targets, the nucleic acid domains of the proximity probes interact, and this interaction leads to the generation of a reporter nucleic acid molecule, for example, by extension and/or ligation. Various PLA and PEA configurations are possible, as described in the art. Nucleic acid domains may be single-stranded or partially double-stranded, or proximity probe sets may be used together with added oligonucleotides to which the nucleic acid domains hybridise.

The nucleic acid domains of the proximity probes in a pair typically are designed to hybridise to one another, or to one or more common oligonucleotide molecules (to which the nucleic acid domains of both proximity probes of a pair may hybridise). Thus, the interaction may be a hybridisation reaction between the nucleic acid domains, or they may both hybridise to a common molecule. A ligation and/or extension product may be generated as an initial reporter nucleic acid molecule, which may be detected directly, or which is amplified to generate amplicons which are detected. Particular mention may be made in this regard of the following patent documents, incorporated herein by reference, which describe proximity assays in which the devices and methods herein may be used: WO 2012/007511; WO 2012/104261; WO 2013/113699; WO/2017/068116; WO 2021/191442; WO 2021/191450; and WO 2022/112300 and EP 1255861. Mention may also be made of the NULISA proximity ligation assay (PLA) of Alamar Bio described by Feng et al. in Nature Communications (2023) 14: 7238 doi: <https://doi.org/10.1038/s41467-023-42834>. These and all other documents mentioned herein are incorporated by reference herein.

The reporter nucleic acid molecule which is generated as the assay reaction product may be detected in any convenient or desired way. This may be any nucleic acid detection method known or available in the art. Conveniently, the reporter nucleic acid molecule, or its amplicon, may be detected by sequencing, and this may include any of the massively parallel, or next generation sequencing methods known or available in the art. Alternatively, the reporter nucleic acid molecule may be detected by a detection probe which hybridises to the reporter molecule. For example, detection methods for detecting reporter molecules include qPCR assays or similar.

For detecting multiple analytes in a sample and/or detecting multiple one or more analytes in multiple samples the devices may be used in detection methods which are carried out in multiplex, i.e. in multiplexed assay methods.

In an embodiment, a multiplexed assay is performed at each reaction space to detect multiple analytes in a single sample. Thus, in such an embodiment, the reagent composition at each reaction space comprises two or more sets of analyte-specific proximity probes (which includes two or more proximity probe pairs), each
5 specific for a different analyte.

To enable the different analytes to be distinguished, the nucleic acid domain of the analyte-specific proximity probe may comprise an ID sequence. This may be a sequence identificatory of, or indicative of, the target analyte in question. In other words, this may be an analyte-specific ID sequence (or in other words a target-specific ID sequence). This can be a barcode sequence, which may for example be
10 detected by sequencing, or it may be detected another way, for example by a specific detection probe (hybridisation probe). The ID sequence may therefore be more broadly defined as a detection sequence. In the case of a proximity probe set (e.g. pair) specific for a given target analyte, the nucleic acid domain of each member of
15 the set or pair may have the same or a different ID sequence. Thus, in one embodiment the reporter nucleic acid molecule may comprise, and may be identified as comprising, a specific combination of ID sequences. Alternatively, the reporter nucleic acid molecule may comprise, and may be identified as comprising, two (or more) copies of the same ID sequence.

In embodiments, each nucleic acid domain of a proximity probe of a matched pair or more may comprise an ID sequence, or a partial identification ID sequence. The reporter molecule which is generated may comprise an ID sequence from each of the matched proximity probes. In other words, the ID sequence of the reporter molecule may be a combination, or composite, of the ID sequences of the individual
25 nucleic acid domains of matched proximity probes.

Thus, an ID sequence in the nucleic acid domain of a proximity probe may become copied (e.g. by an extension reaction templated by the nucleic acid domain), or otherwise incorporated into the reporter nucleic acid molecule (e.g. by ligation), and a reporter nucleic acid molecule corresponding to a particular analyte may be
30 identified by the ID sequence(s) it contains. Depending on the proximity assay configuration, the ID sequence(s) in the reporter may derive from one or more of the nucleic acid domains of the proximity probes. Whilst an ID sequence of a single given proximity probe (or probe set, where all the probes have the same ID sequence) may itself be unique and indicative of the analyte (i.e. analyte-specific), this is not a
35 requirement, and a unique ID sequence may be created in the reporter, through the combination of ID sequences incorporated into it from the nucleic acid domains (e.g. by a ligation or extension reaction). Thus, each reporter nucleic acid molecule which

is generated may comprise a unique ID sequence, or more particularly a unique set or combination of ID sequences, which identifies the analyte.

Put another way, each set of proximity probes may contain or generate an ID sequence (e.g. a barcode sequence). In other words, the matched proximity probes of a set together contain ID sequences which are analyte-specific, or assay-specific (i.e. specific to the assay for a particular analyte).

As noted above, the ID sequences of individual matched proximity probes in a set may be the same or different. In an embodiment, each ID sequence in matched proximity probes is indicative of, or corresponds to, the analyte of interest. However, as explained above, it is not required for a particular ID sequence of an individual proximity probe to be indicative of an analyte of interest – it is the ID sequence of the reporter nucleic acid molecule which is indicative of the analyte of interest. As indicated above, the reporter ID sequence may be a combination or composite. Alternatively, the ID sequence of the reporter nucleic acid molecule may be derived from the nucleic acid domain of a single proximity probe (although it will be understood that interaction of the nucleic acid moieties of matched proximity probes will be required for the reporter nucleic acid molecule to form).

In the case of a device comprising multiple reaction spaces it may be convenient to analyse multiple samples in the same device, or indeed even when the same sample is distributed to multiple reaction spaces, to be able to identify a particular sample aliquot, or particular assay reaction. Thus, it is frequently desired to identify the particular sample, or sample portion or aliquot, which is used in each separate reaction space. To this end the reagent composition may further comprise a sample index oligonucleotide which comprises an ID sequence which identifies the sample, i.e. a sample ID sequence, or sample index sequence, e.g. a sample-specific sequence. In particular, this is a barcode sequence. The sample ID sequence may be unique to a single reaction space, or to a group of reaction spaces, for example, if a sample is distributed to a group of spaces. In an embodiment, the sample ID sequence is used to identify a particular sample. In another embodiment, the sample ID sequence is used to identify the reporter nucleic acid molecule associated with the sample at one particular or given reaction space. The sample index oligonucleotide is the means by which its ID sequence (i.e. the sample index sequence) is incorporated into the reporter nucleic acid molecule (so that it can be detected). Thus, the sample index oligonucleotide may be a primer (for example one of a pair of PCR primers), a ligation adaptor (e.g. an oligonucleotide which is ligated to a nucleic acid domain, or to a reporter nucleic acid molecule or a template

for a ligation or extension reaction. Primers are a convenient way of incorporating a sample index sequence, for example as described in WO 2021/191442, incorporated herein by reference. The Feng paper (supra) describes an alternative mechanism by which a sample index sequence is ligated to a pair of nucleic acid domains of a pair of proximity probes. Thus, the ligation template is partially double-stranded, comprising two single stranded ends and a double-stranded middle portion which comprises the sample index sequence. The two single-stranded ends each bind to a separate nucleic acid domain of the pair, and the two ends are each ligated to the end of the second middle strand. Such ligation templates, or ligation adaptors, are known in the art.

The reagent composition which is dried may further comprise other reagents, notably reagents to perform the assays as described above. These can include reagents such as enzymes (e.g. a DNA polymerase and/or a DNA ligase), or dNTPs for incorporation, primers, adaptors for ligation, ligation templates, additional oligonucleotides etc to generate the reporter nucleic acid molecule.

For example, in one embodiment, the reagent composition may comprise one member of a PCR primer pair, which constitutes the sample index oligonucleotide, and the second member of a PCR primer pair may be supplied or delivered to the device in use. In another embodiment, both members of the PCR primer pair may be included in the dried composition. The PCR primer pair may be used to amplify the reporter nucleic acid molecule in a single PCR step. In another embodiment, a two-step PCR protocol may be used. The primers may be used to incorporate desired sequences into the reporter nucleic acid amplicons, for example, sequencing adaptors e.g. Illumina P5 and P7 sequencing adaptors. Again, this is described in WO 2021/191442.

The reagent composition may comprise one or more internal controls, for controlling various aspects of the assay procedure. Suitable internal controls for use in a PEA are described in Wik *et al.*, 2021, Mol. Cell. Proteomics 20, 100168, incorporated herein by reference.

The reagent composition may comprise an "incubation" control, also referred to as a "control analyte". Absolute quantification of the target analyte may be accomplished by inclusion of known concentration(s) of one or more control analytes and/or referencing the detected level of the target analyte with known control analytes (e.g. through generation of a standard curve). The concentration of the incubation control may be used for quality control of the incubation reaction.

The control analyte may be present in each reaction space at a defined concentration. The control analyte may be added to the reaction space prior to the

5 multiplex detection assay, and detected in each aliquot in the same manner as the other analytes in the sample. In particular, the control analyte may be detected by a set of specific proximity probes. In this way, detection of the control analyte may lead to the generation of a control reporter nucleic acid molecule, specific for the control
10 analyte, in a manner analogous to the generation of an analyte-specific reporter molecule in a test assay reaction. If a control analyte is used, the control analyte is an analyte which cannot be present in the sample of interest. For instance, it may be an artificial analyte, or if the sample is derived from an animal (e.g. a human), the control analyte may be a biomolecule derived from a different species, which is not
15 present in the animal of interest. In particular the control analyte may be a non-human protein. Exemplary control analytes include fluorescent proteins, such as green fluorescent protein (GFP), yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP). The incubation control can be seen as comprising the control analyte together with control-specific proximity probes for its detection.

15 Where the proximity assay is a PEA, the reagent composition may further comprise an "extension" control. This may take the form of a single probe comprising an analyte-binding domain conjugated to a nucleic acid domain which comprises a duplex comprising a free 3' end, which can be extended. The extension control preferably has a structure essentially equivalent to the duplex formed between a pair
20 of proximity probes upon their binding to their target analyte, except it comprises only a single analyte-binding domain. The analyte-binding domain used in the extension control does not recognise an analyte likely to be present in the sample of interest. A suitable analyte-binding domain is an antibody, which may be a commercially available, polyclonal isotype control antibody, such as goat IgG, mouse IgG, rabbit
25 IgG, etc. As such, the extension control may comprise an antibody coupled to a unique pair of oligonucleotides, to keep the oligonucleotides in constant proximity and allow direct hybridisation.

The extension control is used to confirm that the extension step takes place as intended. As such, the extension control may be used for normalisation. Extension
30 of the extension control yields a reporter nucleic acid molecule which may comprise a unique ID sequence, such that it may be identified as the extension control reporter nucleic acid molecule. The extension control may be present at a fixed concentration.

Where the proximity assay comprises a step of amplification, the reagent composition may further comprise an "amplification" control. This will generally be a
35 nucleic acid molecule which resembles, but which may be distinguished from, the reporter nucleic acid molecule, and which may be amplified and identified in a similar manner. For a PEA, the amplification control may comprise a double-stranded DNA

molecule. In particular, the double-stranded DNA molecule may have the same general structure as a reporter nucleic acid molecule generated in the proximity assay. That is to say, the DNA molecule comprises an ID sequence which identifies it as a control reporter nucleic acid molecule. It may further comprise common primer
5 binding sites, shared with all other reporter nucleic acids generated in response to proximity probe set-analyte binding events, to enable binding of the primers used in the amplification reaction(s). Notably, the control DNA molecule may not include sequencing adapters or a sample index – these may be added to the control DNA molecule at the same time as they are added to the reporter nucleic acid molecules
10 generated in response to analyte detection, as described herein (e.g. in PCR amplification).

A double-stranded DNA molecule used as a control in this manner is not only useful in benchmarking analyte concentrations (by comparing their concentrations relative to the control), but it also provides confirmation that reporter nucleic acid
15 molecules generated during analyte detection are amplified, tagged and detected (e.g. by sequencing). In other words, it may be used for quality control during PCR. If the amplification control is not detected when the reporter nucleic acid molecules are analysed (e.g. sequenced), this indicates that the detection method has failed. For instance an amplification step may have failed, or the sequencing reaction may have
20 failed. An amplification control is preferably added to each reaction space prior to performing the multiplex detection assay, for example as part of the reagent composition.

In an embodiment, the reagent composition further comprises a dye. This may not bind to any other reagents in the composition, and may be present in every
25 each reaction space. In this way, the dye may be used for ease of visual inspection, to confirm that the reagent composition is present, particularly in a reaction space, for example the wells of the multiwell plate. For example, the dye may be Brilliant Blue FCF.

By way of representative example, the reagent composition may further
30 comprise one or more of the following:

- (i) one or more enzymes;
- (ii) dNTPs;
- (iii) one or more primers;
- (iv) a ligation template;
- 35 (v) one or more additional oligonucleotides (e.g. for an intra- or inter-molecular ligation reaction templated by the nucleic acid domain(s);
- (vi) one or more internal controls;

- (vii) buffers;
- (viii) salts;
- (ix) a dye; and/or
- (x) blocking agents.

5 In particular embodiments, the enzyme is a polymerase, particularly a DNA polymerase, and/or a ligase.

In an embodiment, the reagent composition does not comprise a DNA polymerase or a DNA ligase.

As indicated above, in use, the detection assay may simply be initiated by
10 introducing a sample into a reaction space of the device. The detection method may involve introducing one or more additional reagents to the reaction space in one or more steps. In some embodiments, all the reagents are present in the reaction space, and no more reagent additions are made.

The method performed in the device, or more particularly the reaction space,
15 may be up to the generation of the reporter molecule, or an amplicon thereof. For detection of the reporter molecule (which includes detection of its amplicon), the contents of the reaction space, or a part thereof may be removed, e.g. for sequencing, or more generally for transfer to another device or instrument where the reporter molecule is detected. This may be achieved manually or may be automated.

20 In an embodiment, the method comprises:

- (i) incubating the sample for a time sufficient to allow the analyte-specific probes to bind to the target analyte;
- (ii) generating reporter nucleic acid molecules comprising the analyte-specific ID sequences;
- 25 (iii) optionally amplifying the reporter nucleic acid molecules;
- (iv) quantitatively detecting the reporter nucleic acid molecules; and
- (v) correlating the quantity of reporter nucleic acid molecules to the quantity of analyte in the sample.

As noted above, the sample may be introduced directly into the reaction
30 reaction space to resuspend the reaction composition, or the sample may itself be resuspended in an aqueous solution and the resuspended sample may be introduced into the reaction space. Alternatively, an aqueous solution may be introduced in the reaction space prior to introduction of the sample.

In a further embodiment of the method, the step of incubating the sample for
35 a time sufficient to allow the analyte-specific probes to bind to the target analyte is performed at room temperature.

All the reagents necessary for step (ii) may be present in the dried composition, or they may be introduced into the reaction step by one or more reagent additions prior to, during or after introduction of the sample.

In an embodiment of the method wherein the dried reagent composition does
5 not comprise a DNA polymerase or DNA ligase, the method may comprise the steps:

- (i) introducing the sample into the reaction space of the device
- (ii) incubating the sample for a time sufficient to allow the analyte-specific probes to bind to the target analyte;
- (iii) introducing a DNA polymerase or ligase into the reaction space to
10 generate a reporter nucleic acid molecule comprising analyte-specific ID sequences;
- (iv) optionally amplifying the reporter nucleic acid molecules;
- (v) quantitatively detecting the reporter nucleic acid molecules; and
- (vi) correlating the quantity of reporter nucleic acid molecules to the
15 quantity of analyte in the sample.

In a further embodiment, such a method comprises adding one or more reagent compositions comprising reagents for performing a nucleic acid amplification reaction, and amplifying the reporter nucleic acid molecules.

In a further embodiment of the method, the dried reagent composition
20 comprises reagents for performing a nucleic acid amplification reaction, the method further comprising amplifying the reporter nucleic acid molecules.

In a further embodiment of the method, the dried reagent composition comprises a sample index oligonucleotide comprising an ID sequence which is unique to the reaction space or a group of reaction spaces and wherein the ID
25 sequence of the sample index oligonucleotide is incorporated into the amplified reporter nucleic acid molecules.

In a further embodiment of the method, the dried reagent composition comprises a synthetic double stranded DNA molecule, and the method comprises:

- (i) Amplifying the reporter nucleic acid molecules and the synthetic
30 double stranded DNA molecule;
- (ii) Quantitatively detecting the amplified synthetic double stranded DNA molecule; and
- (iii) Qualifying the performed amplification reaction as approved if the
synthetic double stranded DNA molecule is detected at or above a
35 predetermined threshold.

As noted above, also provided herein are methods for preparing the devices and the devices produced by such methods.

The devices may be provided as parts of kits containing the device and one or more further components. Such components may include resuspension solutions, e.g. for the dried reagent composition and/or sample, and any reaction reagents or components required or useful for the proximity assay, which are not included in the dried reagent composition. These may include any of the reagents or components discussed above.

For example, such a kit of parts may comprise a device as hereinbefore described and an aqueous solution suitable for resuspension of a sample. In a further embodiment of the kit, the aqueous solution suitable for resuspension of a sample further comprises a surfactant. A further embodiment further comprises a DNA polymerase or a DNA ligase, not incorporated in the pre-dispensed dried reagent composition. A further embodiment further comprises a reagent composition comprising reagents for performing a nucleic acid amplification reaction, not incorporated in the pre-dispensed dried reagent composition. In a further embodiment, the DNA polymerase or DNA ligase is included in the reagent composition comprising reagents for performing a nucleic acid amplification reaction.

The present devices and methods are now illustrated in more detail in the following examples, with reference to the figures.

20

Examples

Example 1 – Exemplary Process for Producing the Dried Reagents for One 96-well Plate Format for Olink® Target 48 Reagents

25

Step 1 – Reagent mixture to be dried

0.3 µL of original forward probes, 0.3 µL of original reverse probes and 2.4 µL of incubation buffer are needed for drying in each well. These are the same amounts as described in the Olink® Target 48 protocol used for incubation with 1 µL of sample.

30

Step 2 – Preparing for reagent mixture and dispensing (calculation based on the amount for each well)

To prepare the reagent mixture for a 10 µL dispensing volume to ensure higher pipetting accuracy, the reagents could be mixed in different ways. An example for dispensing 10 µL in each well (for a 4 µL incubation volume) is set out below.

35

1. Dilute 2.4 μL incubation buffer with MQ water to increase the dispensing volume to ensure higher pipetting accuracy.
2. To protect the probes from vacuum or air drying, 100 mM (50 mM to 300 mM) final concentration of trehalose is added to the diluted probes as a drying stabilizer. Other sugars such as sucrose and glucose may also be used as drying stabilizers, alone or in combination with each other.
3. Dilute 30 μL of forward probes with 470 μL of diluted incubation buffer containing MQ water and stabilizer.
4. Dilute 30 μL of reverse probes with 470 μL of diluted incubation buffer containing MQ water and stabilizer.
5. The diluted probes could be stored for a longer period of time at 4 °C in the fridge.
6. For dispensing, mix 500 μL of diluted forward probes and 500 μL of diluted reverse probes. Dispense 10 μL in each well in a 96-well plate.

Tests have also been performed adding Tween 80 to the stabilizer at 5-10 mg/ml to some assay panels.

The dispensing volumes can be varied depending on the minimum volume that the dispensing pipette can handle whilst maintaining accuracy.

Step 3 – Drying of the reagents

After the reagents are dispensed, they are dried with air drying for overnight (Results shown in Figures 2 and 3) or in a vacuum chamber for 2 hours at 35 °C, for example (Figure 4). The drying time and temperature may vary depending on the concentration of stabilizer used.

Step 4 – Protection of the dried reagents

The dried reagents are heat sealed by metal membrane to protect it from humidity and dust. A vacuum sealing bag containing a silica gel bag is used as a second protective layer for further protection from humidity.

Step 5 – Immunoassay

The sample is diluted by 1% BSA in a 1:3 ratio of sample to 1% BSA. 4 μL of the diluted plasma samples is then added to the dried reagents.

Step 6 – PEA

A routine protocol may be used for PEA, e.g. as described in Assarsson et al., PLoS ONE 9(4): e95192.

Example 2 – Exemplary Process for Producing the Dried Reagents for One 96-well Plate Format for sequencing readout

For protocols which use sequencing as a readout (e.g. as described in Wik et al., 2021, Mol Cell Proteomics 20, 100168, incorporated herein by reference), different sample indices (e.g. at a concentration of 1 μ M) are added to each well of the 96-well plates during Step 1 of the process in Example 1.

In Step 6 of the process in Example 1, a one-step PCR may be used to run the PEA and incorporate the sample indices into the PEA product, or the 2-step PCR described in Wik et al., where the extension products in the different abundance blocks are first amplified to saturation (PCR 1 in Wik et al.) and pooled, prior to addition of index primers to incorporate sample specific information into the amplicons (PCR 2 in Wik et al.). The one-step PCR resembles PCR 2 but is performed individually for the different abundance blocks using the same set of sample specific index primers for each abundance block.

The sequencing protocol described in the protocol for the Explore panel is then followed.

Example 3 - Comparison between Results Generated from the Standard Protocol versus the Protocol Based on Dried Reagents

The performance of the protocol based on dried reagents was compared to that of the standard protocol (wet reagents and 4°C overnight incubation) across 15 different samples. In this experiment, for the protocol based on dried reagents, 3.5 μ L of probe mixture for drying, 100 mM trehalose and 5mg/ml Tween 80 were used,

The average S/N values of all assays for different samples were shown to be very similar between the two protocols (Figure 2A). The high R-square value ($R^2=0.9814$) suggests a high correlation between the two protocols for all assays (Figure 2B).

Besides the S/N, the NPX were also calculated from the same experiment. Intra- and inter-plate CV% were compared between the two protocols based on linear NPX values. The two protocols showed similar intra- and inter-plate CV% between the protocol based on New protocol (dried reagents + RT overnight incubation) and the standard protocol (wet reagents + 4°C overnight incubation). Results are shown in Table 2 below.

Table 2

		CCL 8	IL33	CXCL 12	OLR 1	IL27	IL2	CXC L9	TGF A	IL1B
Intra-plate CV%	New	3%	5%	10%	3%	10%	7%	4%	3%	5%
	Standard	5%	6%	8%	5%	8%	7%	5%	3%	5%
Inter-plate CV%	New	7%	6%	16%	3%	13%	8%	6%	4%	6%
	Standard	2%	5%	8%	5%	7%	7%	2%	3%	8%
		IL6	IL4	TNF SF12	TSL P	CCL 11	HGF	FLT 3LG	IL17 F	IL7
Intra-plate CV%	New	4%	10%	3%	7%	4%	5%	3%	6%	4%
	Standard	5%	6%	5%	5%	4%	5%	4%	8%	4%
Inter-plate CV%	New	5%	7%	4%	8%	4%	6%	4%	5%	3%
	Standard	4%	4%	2%	2%	1%	1%	2%	5%	2%
		IL13	IL18	CCL1 3	TNF SF 10	CXC L10	IFN G	IL10	CCL 19	TNF
Intra-plate CV%	New	5%	3%	5%	4%	5%	7%	7%	8%	4%
	Standard	4%	3%	4%	3%	6%	6%	7%	5%	4%
Inter-plate CV%	New	10%	5%	7%	5%	9%	6%	8%	6%	6%
	Standard	6%	2%	3%	2%	4%	4%	3%	3%	6%
		IL15	CCL 3	CXCL 8	MM P12	CSF 2	CSF 3	VEG FA	IL17 C	EGF
Intra-plate CV%	New	4%	5%	4%	5%	4%	4%	5%	5%	5%
	Standard	6%	6%	5%	5%	5%	3%	5%	5%	6%
Inter-plate CV%	New	3%	7%	9%	23%	10%	3%	8%	7%	8%
	Standard	7%	3%	7%	19%	3%	2%	2%	3%	2%
		CCL 2	IL17 A	OSM	CSF 1	CCL 4	CXC L11	LTA	CCL 7	MM P1

Intra-plate CV%	New	3%	7%	5%	3%	6%	3%	2%	2%	4%
	Standard	4%	8%	4%	5%	6%	5%	4%	5%	5%
Inter-plate CV%	New	6%	11%	6%	5%	11%	6%	6%	6%	6%
	Standard	2%	3%	3%	2%	3%	2%	2%	1%	5%
		Average								
Intra-plate CV%	New	5%								
	Standard	5%								
Inter-plate CV%	New	7%								
	Standard	4%								

Beneficial effects of including Tween 80 as a stabilizer in some assay panels have also been observed.

5 Example 4 – Use of Sample Control for quality control of the assays

The Sample Control is a pooled human plasma sample, with spiked in recombinant antigens for some proteins, to ensure that all 45 proteins are detected. To evaluate how the protocol using dried reagents affected each individual assays, the sample control was used as the sample. In this experiment, for the protocol based on dried reagents, 3.5 µL of probe mixture for drying, 100 mM trehalose and 5mg/ml Tween 80 were used. No assays were undetected by the protocol based on dried reagents (Figure 3A). The high R-square value ($R^2=0.9315$) suggests a high correlation between the two protocols for all assays (Figure 3B). The results suggest that the data for the protocol based on air dried reagents and the standard protocol are similar and highly correlated for most of the assays.

Example 5 – Comparison between Results Generated from the Standard Olink® Target 48 (T48) Protocol versus the Protocol Based on Vacuum Dried T48 Reagents

The performance of the protocol based on vacuum-dried reagents was tested using T48 reagents and compared to the results generated from the standard T48 protocol (wet reagents) across 48 different assays. 10 µL of further diluted probes

and 100 mM of trehalose in the 10 µL were used in this experiment, but Tween 80 was not used.

5 The wet and dried reagents generated similar signal to noise ratios (S/N) for all assays except one (Figure 4A) and the high R-square value ($R^2=0.9686$) suggests a high correlation between the two protocols for each assay in the T48 panel (Figure 4B). The results therefore suggest that the performance of the protocol based on dried T48 reagents is similar to that of the standard T48 protocol, which is based on wet reagents. In other words, vacuum-drying of the reagents have not negatively
10 affected performance of the assays.

Two tests were also performed to mimic the production process and vacuum drying. Results show that the dried reagents had similar or mostly likely improved CV%. (*data not shown*).

15

Example 6 – Comparison between Results Generated from the Standard Olink® Explore Protocol versus the Protocol Based on Corresponding Dried Reagents

The performance of the protocol based on vacuum-dried reagents was tested with a panel of probes, with NGS readout and compared to the results generated from the standard Olink® Explore protocol (wet reagents). In line with Example 2, the corresponding reagents were also dried with the sample index primers. 10 µL of further diluted probes and 100 mM of trehalose in the 10 µL were used in this experiment, but Tween 80 was not used.

25 Similar but slightly lower S/N results were generated for the dried reagents compared to the wet reagents, reflected by the linear fit vs $X=Y$ line fitting (Figure 5). The wet and dried reagents generated quite a high S/N correlation for the different assays, as shown by the high R-squared value ($R^2 = 0.92$) (Figure 5). The results therefore suggest that the performance of the protocol based on dried reagents is similar or
30 only slightly lower than that of the standard protocol, which is based on wet reagents. In other words, vacuum-drying of the reagents have not significantly affected the performance of the assays.

Claims

1. A device comprising at least one reaction space with a single pre-dispensed dried reagent composition suitable for performing an assay method for detecting at least one analyte in a sample to be added to the reaction space, wherein the reagent composition comprises at least one set of analyte-specific proximity probes and a drying stabiliser, wherein the proximity probes in each set comprise a binding domain for binding directly or indirectly to the analyte and a nucleic acid domain.
2. The device of claim 1, wherein the reaction space is an area or a reaction container.
3. The device of claim 1 or claim 2 comprising multiple separate reaction spaces.
4. The device of any one of claims 1 to 3, wherein the device is a multi-well plate, an array or a microfluidic device.
5. The device of any one of claims 1 to 4, wherein the reaction space is configured to receive a volume of no more than 10 µl.
6. The device of any one of claims 1 to 5, wherein the reagent composition:
 - (i) comprises a pair of analyte-specific proximity probes; or
 - (ii) comprises a pair of proximity probes for a proximity extension assay (PEA) or proximity ligation assay (PLA).
7. The device of any one of claims 1 to 6, wherein the reagent composition at each reaction space comprises two or more sets of analyte-specific proximity probes, each set specific for a different analyte, and wherein the nucleic acid domain of at least one proximity probe of a set comprises an ID sequence, to allow generation of a reporter nucleic acid molecule comprising an analyte-specific ID sequence.
8. The device of any one of claims 1 to 7, wherein the drying stabiliser comprises one or more sugars or sugar alcohols.
9. The device of claim 8, wherein the drying stabiliser comprises trehalose, glucose, sucrose and/or mannitol.

10. The device of any one of claims 1 to 9, wherein the reagent composition further comprises a sample index oligonucleotide comprising an ID sequence which is unique to the reaction space or a group of reaction spaces.
- 5 11. The device of claim 10, wherein the sample index oligonucleotide is a primer, a ligation adaptor, or a ligation and/or extension template.
12. The device of any one of claims 1 to 11, wherein the reagent composition further comprises a dye.
- 10 13. The device of any one of claims 1 to 12, wherein the reagent composition further comprises a synthetic double-stranded DNA molecule.
14. The device of any one of claims 1 to 13, wherein the reagent composition further comprises a blocking agent.
- 15 15. The device of any one of claims 1 to 14, wherein the reagent composition does not comprise a DNA polymerase or a DNA ligase.
- 20 16. The device of any one of claims 1 to 14, wherein the reagent composition further comprises one or more reagents for generation of a reporter nucleic acid molecule, for amplification, and/or for detection of a reporter nucleic acid molecule.
- 25 17. The device of claim 16, wherein the reagent composition further comprises one or more of the following:
- (i) one or more enzymes;
 - (ii) dNTPs;
 - (iii) one or more primers;

30 (iv) a ligation template;

 - (v) buffers;
 - (vi) a sequencing adaptor; and/or
 - (vii) salts.
- 35 18. The device of claim 17, wherein the enzyme is a DNA polymerase and/or a DNA ligase.

19. A method of performing a detection assay to detect a target analyte in a sample, said method comprising introducing a sample into a reaction space of a device as defined in any one of claims 1 to 18.
- 5 20. The method according to claim 19, wherein the reagent composition at each reaction space comprises two or more analyte-specific proximity probe pairs, each pair specific for a different analyte, and wherein the nucleic acid domain of each analyte-specific proximity probe comprises an analyte-specific ID sequence, wherein the method comprises:
- 10 i. Incubating the sample for a time sufficient to allow the analyte-specific probes to bind to the target analyte;
- ii. Generating reporter nucleic acid molecules comprising the analyte-specific ID sequences;
- iii. Optionally amplifying the reporter nucleic acid molecules;
- 15 iv. Quantitatively detecting the reporter nucleic acid molecules; and
- v. Correlating the quantity of reporter nucleic acid molecules to the quantity of analyte in the sample.
21. The method according to claim 19 or 20, wherein the sample is resuspended in an aqueous solution and the resuspended sample is introduced into the reaction space.
22. The method according to claim 19 or 20, wherein an aqueous solution is introduced into the reaction space and the sample is resuspended in the aqueous solution after introduction into the reaction space.
- 25 23. The method according to any one of claims 21 or 22, wherein the aqueous solution comprises a surfactant.
- 30 24. The method according to any one of claims 19 to 23, wherein the step of incubating the sample for a time sufficient to allow the analyte-specific proximity probes to bind to the target analyte is performed at room temperature.
25. The method according to any one of claims 19 to 24, comprising the steps:
- 35 i. Introducing the sample into the reaction space of a device as defined in claim 15;

- ii. Incubating the sample for a time sufficient to allow the analyte-specific proximity probes to bind to the target analyte;
 - iii. Introducing a DNA polymerase or ligase into the reaction space to generate a reporter nucleic acid molecule comprising analyte-specific ID sequences;
 - iv. Optionally amplifying the reporter nucleic acid molecules;
 - v. Quantitatively detecting the reporter nucleic acid molecules; and
 - vi. Correlating the quantity of reporter nucleic acid molecules to the quantity of analyte in the sample.
26. The method according to any one of claims 19 to 25, comprising adding one or more reagent compositions comprising reagents for performing a nucleic acid amplification reaction, and amplifying the reporter nucleic acid molecules.
27. The method according to any one of claims 19 to 25, wherein the dried reagent composition comprises reagents for performing a nucleic acid amplification reaction, the method further comprising amplifying the reporter nucleic acid molecules.
28. The method according to any one of claims 20 to 27, wherein the dried reagent composition comprises a sample index oligonucleotide comprising an ID sequence which is unique to the reaction space or a group of reaction spaces and wherein the ID sequence of the sample index oligonucleotide is incorporated into the amplified reporter nucleic acid molecules.
29. The method according to any one of claims 20 to 28, wherein the dried reagent composition comprises a synthetic double-stranded DNA molecule, and wherein the method comprises:
- i. Amplifying the reporter nucleic acid molecules and the synthetic double stranded DNA molecule;
 - ii. Quantitatively detecting the amplified synthetic double stranded DNA molecule; and
 - iii. Qualifying the performed amplification reaction as approved if the synthetic double stranded DNA molecule is detected at or above a predetermined threshold.

30. A method of preparing a device comprising at least one reaction space with a single pre-dispensed dried reagent composition, wherein the reagent composition is as defined in any one of claims 1 or 6 to 18, said method comprising introducing a volume of liquid comprising the reagent composition to the reaction space, and drying said liquid volume to form a dried reagent composition.
31. The method according to claim 30, wherein the drying is air-drying.
32. The method according to claim 30, wherein the drying is vacuum-drying.
33. A device obtainable through the method according to any one of claims 30 to 32.
34. A kit of parts comprising a device according to any one of claims 1-18 or 33 and an aqueous solution suitable for resuspension of a sample.
35. The kit of parts according to claim 34, wherein the aqueous solution suitable for resuspension of a sample further comprises a surfactant.
36. The kit of parts according to claim 34 or 35, further comprising a DNA polymerase or a DNA ligase, not incorporated in the pre-dispensed dried reagent composition.
37. The kit of parts according to any one of claims 34 to 36, further comprising a reagent composition comprising reagents for performing a nucleic acid amplification reaction, not incorporated in the pre-dispensed dried reagent composition.