

An Air Dried Composition

Technical Field

The present invention relates to an air dried composition. Specifically, the present invention relates to an air dried composition that maintains the biological activity of the compounds upon re-solubilization after a certain storage time. The air dried composition contains a polymerase for use in polymerase chain reaction after re-solubilization. The present invention also relates to a method for preparing the air dried composition, and a kit for PCR reaction comprising the air dried composition.

Background

Biological reaction compounds are usually stable in solubilized condition for a period of time depending on the storage condition. An enormous amount of work was performed to prolong the life-span of biological reaction compounds in the past. Among the most efficient method is to store the biological reaction compounds in dry form. However, the methods of drying liquid reaction mixtures are often complex and the drying procedures are expensive and laborious. Freeze drying or lyophilization is a well-established technique ~~towards~~ used for storage of protein ~~that is~~ mentioned in literatures. However, both these techniques are expensive. On the other hand, air drying is more economical and it can be divided into three processes: natural air drying, hot air drying and, imitation-natural air drying. Hot air drying is utilised in many industrial processes to maintain the stability of product quality and output. A precision oven with a blower is used to dry the liquid at a temperature higher than room temperature (i.e., drying above 50 °C for 80 minutes).

One of the most commonly used reagent in In Vitro diagnosis (IVD) is the Taq DNA Polymerase, the main ingredient in any Polymerase Chain Reaction (PCR) reaction. Typically, PCR reagents are stored in glycerol solution which must be maintained at temperatures below room temperature. Lyophilisation or freeze drying is a process widely used in the preparation of reagents for nucleic acid analysis and other biological processes because it allows for long term stability of otherwise labile biomolecules, and provides a convenient method of storage, shipping and reconstitution. However, there are numerous technical challenges involved in producing a lyophilised or freeze-dried reagent for PCR analysis. Current technology for producing dry biological reagent compositions involves

procedures such as dry-blending, spray-drying, freeze-drying, fluidized bed drying, and/or cryogenic freezing. All of these procedures have limitations and drawbacks including consistency and reliability. During drying of a liquid solution comprising different components, the concentration of the components increases and drastically changes the properties of the components which may lead to a less efficient PCR reaction.

Therefore, there is a need for providing a formula that can withstand the heat stress and overcome issues related to sub-optimal storage condition and preserve the reagent mixture as it is exposed to high temperature and dehydration.

Summary

In the first aspect, the present invention provides an air dried composition, comprising nucleotides, a polymerase and one or more stabilizing molecules. In an embodiment, the nucleotides are dNTP. In an embodiment, the polymerase is Taq DNA polymerase. According to the present invention, the air dried composition maintains the biological activity of the compounds upon re-solubilization after a certain storage time. According to the present invention, the air dried composition is obtained by air drying a composition comprising the nucleotides, the polymerase and one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air. In an embodiment, the air dried composition is obtained by air drying said composition in a conventional oven with circulating air for 20 to 40 minutes, preferably 20 to 30 minutes, preferably 30 minutes, at temperature between 50 to 80 °C, preferably at temperature between 55 to 75 °C, more preferably at temperature of 75 °C. In an embodiment, the air dried composition is obtained by air drying said composition in a conventional oven with circulating air for 30 minutes at temperature of 75 °C. The time required to dry said composition will correlate with and can be varied depending on the drying temperature used.

According to the present invention, the stabilizing molecules are selected from the groups consisting of a protein, carbohydrates, a neutral chemical compound, an organic solvent, a diol, a quaternary ammonium salt, an anionic polymer, an amino acid, PEG, and combinations thereof. In an embodiment, the stabilizing molecules are a combination of a protein, a carbohydrate, a neutral chemical compound, an organic solvent, a diol, a quaternary ammonium salt, and any one of an anionic polymer, an amino acid and PEG.

In an embodiment, the protein is Bovine Serum Albumin (BSA). In an embodiment, the carbohydrates are selected from the groups consisting of trehalose and raffinose. In an embodiment, the neutral chemical compound is betaine. In an embodiment, the organic solvent is DMSO. In an embodiment, the diol is 1,2-propanediol (1,2-PPD). In an embodiment, the quaternary ammonium salt is tetramethylammonium chloride (TMAC). In an embodiment, the anionic polymer is dextran. In an embodiment, the amino acid is L-threonine.

10 In an embodiment, the stabilizing molecules are a combination of BSA, trehalose, raffinose, betaine, DMSO, 1,2-propanediol, tetramethylammonium chloride, and any one of dextran, L-threonine and PEG.

In an embodiment, the protein is present in a concentration of 3.7 to 37 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, the carbohydrates are present in a concentration of 1 to 300 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. For example, trehalose is present in a concentration of 100 to 300 mM, preferably 150 mM, and raffinose is present in a concentration of 1 to 10 mM, preferably 5 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, the neutral chemical compound is present in a concentration of 100 to 400 mM, preferably 400 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, the organic solvent is present in 10% (v/v) in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, the diol is present in a concentration of 100 to 300 mM, preferably 100 to 150 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, the quaternary ammonium salt is present in a concentration of 25 to 75 mM, preferably 25 to 50 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, if any, the anionic polymer is present in a concentration of 1 to 10 mM, preferably 1 to 5 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing

molecules prior to drying. In an embodiment, if any, the amino acid is present in a concentration of 10 to 40 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, if any, PEG is present in a concentration of 1 to 10 mM, preferably 1 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying.

In an embodiment, BSA is present in a concentration of 3.7 to 37 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, trehalose is present in a concentration of 100 to 300 mM, preferably 150 mM, in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, raffinose is present in a concentration of 1 to 10 mM, preferably 5 mM, in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, betaine is present in a concentration of 100 to 400 mM, for example 400mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, DMSO is present in 10% (v/v) in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, 1,2-PPD is present in a concentration of 100 to 300 mM, preferably 100 to 150 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, TMAC is present in a concentration of 25 to 75 mM, preferably 25 to 50 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, if any, dextran is present in a concentration of 1 to 10 mM, preferably 1 to 5 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, if any, L-threonine is present in a concentration of 10 to 40 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying. In an embodiment, if any, PEG is present in a concentration of 1 to 10 mM, preferably 1 mM in the composition comprising the nucleotides, the polymerase and one or more stabilizing molecules prior to drying.

In an embodiment, the one or more stabilizing molecules are a combination of a protein in a concentration of 3.7 to 37 mM, a carbohydrate in a concentration of 1 to 300 mM, a neutral chemical compound in a concentration of 100 to 400 mM, preferably 400 mM, an organic solvent in a concentration of 10% (v/v), a diol in a concentration of 100 to 300 mM, preferably 100 to 150 mM, a quaternary ammonium salt in a concentration of 25 to 75 mM, preferably 25 to 50 mM, and any one of an anionic polymer in a concentration of 1 to 10 mM, preferably 1 to 5 mM, an amino acid in a concentration of 10 to 40 mM and PEG in a concentration of 1 to 10 mM, preferably 1 mM.

10 In an embodiment, the one or more stabilizing molecules are a combination of BSA in a concentration of 3.7 to 37 mM, trehalose in a concentration of 100 to 300 mM, preferably 150 mM, raffinose in a concentration of 1 to 10 mM, preferably 5 mM, betaine in a concentration of 100 to 400 mM, preferably 400 mM, DMSO in a concentration of 10% (v/v), 1,2-propanediol in a concentration of 100 to 300 mM, preferably 100 to 150 mM, 15 tetramethylammonium chloride in a concentration of 25 to 75 mM, preferably 25 to 50 mM, and any one of dextran in a concentration of 1 to 10 mM, preferably 1 to 5 mM, L-threonine in a concentration of 10 to 40 mM and PEG in a concentration of 1 to 10 mM, preferably 1 mM.

20 In an embodiment, the one or more stabilizing molecules are a combination of 3.7 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 150 mM 1,2-propanediol, 25 mM tetramethylammonium chloride, 10 mM L-threonine, and 1 mM PEG.

25 In an embodiment, the one or more stabilizing molecules are a combination of 3.7 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 100 mM 1,2-propanediol, 25 mM tetramethylammonium chloride, 5 mM dextran, and 1 mM PEG.

In an embodiment, the one or more stabilizing molecules are a combination of 37 30 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 150 mM 1,2-propanediol, 50 mM tetramethylammonium chloride, 1 mM dextran, and 40 mM L-threonine.

In the second aspect, the present invention provides a method for preparing the air dried composition according to the present invention, which comprises air drying a composition comprising nucleotides, a polymerase and one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air. In an embodiment, the nucleotides are dNTP. In an embodiment, the polymerase is Taq DNA polymerase. In an embodiment, the air dried composition is obtained by air drying said composition in a conventional oven with circulating air for 20 to 40 minutes, preferably 20 to 30 minutes, preferably 30 minutes, at temperature between 50 to 80 °C, preferably at temperature between 55 to 75 °C, more preferably at temperature of 75 °C. In an embodiment, the air dried composition is obtained by air drying said composition in a conventional oven with circulating air for 30 minutes at temperature of 75 °C. The time required to dry said composition will correlate with and can be varied depending on the drying temperature used. The stabilizing molecules described above in the first aspect can be used in this aspect.

In an embodiment, primers can be added after the air drying process is completed. Addition of primers after the air drying process increases the wider usage of this product. In principle, any fluorescence probes are compatible with the air dried composition according to the present invention. In an embodiment, the fluorescence probe is ROX.

The drying method according to this invention is cheaper and simpler without the requirement of extensive trainings. The air dried composition according to the present invention is subject to real-time stability and accelerated aging, and the results show that the air dried composition according to the present invention can be stored at room temperature ($\pm 25^{\circ}\text{C}$) RH <60% for 48 days without compromising the performance of the enzyme activity, rendering the possibility of transportation at room temperature and the inexpensive cost of delivery.

In the third aspect, the present invention provides a kit for PCR reaction, comprising the air dried composition according to the present invention. As described above, the air dried composition according to the present invention comprises nucleotides, a polymerase and one or more stabilizing molecules. In an embodiment, the nucleotides are dNTP. In an embodiment, the polymerase is Taq DNA polymerase.

Brief Description of the Drawings

To clearly indicate the technical solution of the present invention, a brief introduction thereto is provided below in reference to the Figures. Apparently, these Figures are merely some embodiments recorded in this application. The present invention includes
5 but is not limited to these Figures.

Fig. 1 shows three potential formulations successfully amplified target DNA in ROX-channel (commercially available master mix = square, master mix plus different combinations of additives = circle, triangle and cross).

Fig. 2 shows a summary of the steps required to air dry and evaluation of master mix
10 in a batch process.

Fig. 3 shows a representative graph of air dried master mix (triangle), a non-air-dried (circle) master mix with additives, and of Taq polymerase without additive (square).

Fig. 4 shows linear regression analysis of air dried master mix after 39 days of accelerated aging at 37 °C. X-axis shows the sampling time points T1 – T11.

15 Detailed Description

As used herein, “an air dried composition” is obtained by air drying a composition comprising nucleotides, a polymerase and one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air, which is interchangeably used with “an air dried mixture”, “a dried mixture”, “a dried composition”, “a dry mixture”, or a “dry
20 composition”.

The present invention has employed a specific strategy to improve the stability of a PCR master mix while maintaining the amplification performance. Several groups of molecules are used as stabilizing molecules in a PCR master mix. As described above, the
25 stabilizing molecules are selected from the groups consisting of a protein, carbohydrates, a neutral chemical compound, an organic solvent, a diol, a quaternary ammonium salt, an anionic polymer, an amino acid, PEG, and combinations thereof.

As the main ingredient in a PCR reaction is a polymerase such as a Taq DNA
30 polymerase, enzyme stabilizers are added. Such stabilizing molecules include a protein such as BSA. BSA has been known to increase enzyme activity, thereby improving assay robustness and reproducibility.

Without wishing to be bound by theory, carbohydrates such as trehalose and raffinose act as cryoprotectants in the air dried composition according to the present invention. These carbohydrates are chosen for their high T_g (glass transition temperature) properties.

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Without wishing to be bound by theory, a quaternary ammonium salt such as TMAC is used as a catalyst due to its thermal stability property and possibility of increasing amplification sensitivity; neutral chemical compounds such as betaine can reduce DNA melting temperature and facilitate strand separation and are commonly added into PCR master mixes; and an organic solvent such as DMSO can disrupt base pairing and prevent intramolecular stem loops in GC-rich templates. Therefore, the quaternary ammonium salt such as TMAC, the neutral chemical compound such as betaine, and the organic solvent such as DMSO are added to improve PCR amplification with GC-rich templates or templates difficult to be amplified.

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Without wishing to be bound by theory, an anionic polymer such as dextran and PEG are added as a molecular crowding agent to speed up PCR reactions by increasing efficiency of the polymerase activity; a diol such as 1,2-PPD is added into PCR master mix to neutralize PCR inhibitors; and an amino acid such as L-threonine is added to improve the stability of the fluorophore added into the master mix.

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Without the addition of any stabilizing molecules, the polymerase activity was greatly reduced after drying even when stored at the recommended temperature, i.e., 4 °C. Consequently, the requirements to stabilize the glycerol free Taq DNA polymerase for drying increased. In the present invention, the addition of all the additives (listed in table 2) enables the glycerol free Taq DNA polymerase to be dried at high temperature (for example, 70 to 80 °C) and, transport at ambient temperature.

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Examples

To further understand the present invention, preferable solutions of the present invention are described in details below in reference to examples. However, these examples are merely used to illustrate the characteristics and merits of the present invention, but not to limit the protection scope of the present invention.

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Example 1 Screening of a cocktail of additives that successfully amplified the targeted template

Based on literature reviews, a total of ten additives, namely, trehalose, Betaine, DMSO, TMAC, 1,2-PPD, BSA, raffinose, dextran, L-threonine and PEG, was selected to be tested. To determine the individual effects of various factors (additive) that can influence the output results of the measurement, minitab software were used to design the experiment (DoE).

All the additives used in this invention were of analytical grade. Working stock for each additive was prepared and stored according to the safety data sheet and kept at recommended condition until use. A total of 130 combinations of stabilizing compounds was then prepared according to Table 1, and added to a qPCR master mix. Then, qPCR was carried out to evaluate the amplification performance.

The components of qPCR master mix include Taq Buffer, MgCl₂, Taq polymerase enzyme. The additives (items 6-15) and the amounts thereof per reaction when they are used are stated in Table 1 below.

Table 1 The components in qPCR master mix and the additives used.

Item	Component	Amount per reaction
1	10x <i>Taq</i> Buffer	10%
2	MgCl ₂ (25 mM)	2.5 - 5 mM
3	<i>Taq</i> Polymerase (Hot start)	5U
4	20X Gene specific forward & reverse primer, probe, dNTP mix	200-400nM
5	Tris-HCL pH8.8	25mM
	Additives:	
6	Trehalose	100-300mM
7	1,2-PPD	100-300mM
8	TMAC	25-75mM
9	Betaine	100-400mM

10	BSA	3.7-37mM
11	DMSO	10% (v/v)
12	Raffinose	1-10mM
13	Dextran	0-10mM
14	L-threonine	0-40mM
15	PEG	0-10mM

Formulations that successfully amplified the targeted template are shown in Figure 1, are then repeated and used for next step.

- 5 As shown in Figure 1, three potential formulations successfully amplified target DNA in ROX-channel. PCR was performed on Bio-Rad CFX96 Real-Time PCR system. In Figure 1, square represents a commercially available master mix (as control), and circle, triangle and cross represent master mix plus different combinations of additives as shown in Table 2 below, respectively.

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Table 2 The combinations of additives which successfully amplified target DNA.

	<i>Cocktail 1 (circle)</i>	<i>Cocktail 2 (triangle)</i>	<i>Cocktail 3 (cross)</i>
<i>Trehalose</i>	<i>150mM</i>	<i>150mM</i>	<i>150mM</i>
<i>1,2-PPD</i>	<i>150mM</i>	<i>100mM</i>	<i>150mM</i>
<i>TMAC</i>	<i>25mM</i>	<i>25mM</i>	<i>50mM</i>
<i>Betaine</i>	<i>400mM</i>	<i>400mM</i>	<i>400mM</i>
<i>BSA</i>	<i>3.7mM</i>	<i>3.7mM</i>	<i>37mM</i>
<i>DMSO</i>	<i>10%</i>	<i>10%</i>	<i>10%</i>
<i>Raffinose</i>	<i>5mM</i>	<i>5mM</i>	<i>5mM</i>
<i>Dextran</i>	<i>0mM</i>	<i>5mM</i>	<i>1mM</i>
<i>L-threonine</i>	<i>10mM</i>	<i>0mM</i>	<i>40mM</i>
<i>PEG</i>	<i>1mM</i>	<i>1mM</i>	<i>0mM</i>

Example 2 Preparation of air dried master mixes

A cocktail of additives was added to the qPCR master mix, and a dry master mix was prepared by drying the master mix in a conventional oven with circulating fan such as MeMmert Oven UN75, as shown in Figure 2.

5 With respect to the drying procedure, several workflows were tested. The flap of the oven was set to 10%, 20% and 30% and fan speed to 100% to achieve a moisture lost between 70-90% in 20 to 40 minutes. The time required to dry the liquid mixture will correlate with the drying temperature used.

10 Within the present invention, it was shown that the master mix can be dried at temperature of 75 °C for 30 minutes without significant loss of activity. This condition is used in the following Examples.

Example 3 Evaluation of the amplification performance of the air dried master mixes in qPCR

15 Master mix plus additives and primers were prepared and aliquoted into PCR tubes and half of the samples are air-dried at 75 °C for 30 minutes while the other half of the samples remain wet and stored at 4 °C. However, primers can also be added after the drying process is completed if needed. Addition of primers after drying process increases the wider
20 usage of this product. In principle, any fluorescence probes are compatible with the air dried master mix. Within this invention however, only a single fluorescence probe – ROX was used in all experiments.

25 Once drying process is completed, both (dried and wet master mixes) were added with DNA template in nuclease free water to make up to 20 µL reaction and PCR reaction was carried out using the same PCR condition. Dried master mix form a “glassy” appearance under ambient temperature. A proper mixing of the PCR mixtures is achieved by either pipetting up-and-down for 30 times or vortex for 30 sec.

30 Figure 3 shows representative graph of air dried master mix with additive (triangle) and of non-air-dried master mix (circle) with additives (Cocktail 2) compared to Taq polymerase without additive (square). PCR reaction was performed with actin primer on human genomic DNA (Takara) on Applied Biosystems QuantStudio™ Real-Time PCR

system. As shown in Figure 3, the performance of air dried master mix surpasses the non air dried master mix and the Taq polymerase without additives.

The air dried master mixes is prepared by adding Cocktail 2 shown in Table 2 and air dried at a temperature of 75 °C for 30 minutes as follows:

1. Pre-heat the oven to 80 °C (allow the temperature to stabilise for 30 mins before using the oven for drying).
2. Set the air flap of the oven to 20% and fan speed to 100%.
3. Vortex each reagent for 30 sec and do a quick-spin prior to aliquoting for use.
4. Aliquote 5uL of master mix (10x Taq Buffer, 25 mM MgCl₂, Taq Polymerase, 20X Gene specific forward & reverse primer, probe, dNTP mix, Tris-HCL pH8.8, and Cocktail 2 into 8-well PCR-tubes and air-dry the tubes at 75 °C for 30 mins.
5. Remove the tubes with dried master mix from the oven and sealed the tubes in aluminium pouch with sachet of silica gel.

Example 4 Evaluation of the stability of the air dried master mixes

The stability of the air dried master mix prepared according to Example 2 with Cocktail 2 shown in Table 2 was evaluated by accelerated aging. With an estimated target shelf-life of 180 days if stored at 4 °C, the air dried master mixes were kept at 37 °C for 39 days (based on Q10, accelerated aging factor 9.85). Both the air dried master mix as well as the wet master mix were tested in duplicate at 4 days intervals to evaluate the degree of activity loss, if any. The average CT of the air dried master mixes was compared to the average CT of the wet master mix.

Our preliminary studies suggest that, no significant difference between the air dried master mixes and wet master mix where the average CT remains stable after storage at 25 °C for 48 days which is equivalent to 202 days if stored at 4 °C. In addition, the air dried master mix is stable up to 48 days at room temperature (± 25 °C) RH <60%, as shown in Figure 4.

CLAIMS

1. An air dried composition comprising nucleotides, a polymerase and one or more stabilizing molecules.

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2. The air dried composition according to claim 1, wherein the nucleotides are dNTP.

3. The air dried composition according to claim 1, wherein the polymerase is a Taq DNA polymerase.

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4. The air dried composition according to any one of claims 1 to 3, wherein the one or more stabilizing molecules are selected from the group consisting of a protein, a carbohydrate, a neutral chemical compound, an organic solvent, a diol, a quaternary ammonium salt, an anionic polymer, an amino acid, PEG, and any combination thereof; preferably, a combination of a protein, a carbohydrate, a neutral chemical compound, an organic solvent, a diol, a quaternary ammonium salt, and any one of an anionic polymer, an amino acid and PEG; more preferably, a combination of BSA, trehalose, raffinose, betaine, DMSO, 1,2-propanediol, tetramethylammonium chloride, and any one of dextran, L-threonine and PEG.

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5. The air dried composition according to claim 4, wherein the protein is BSA.

6. The air dried composition according to claim 4, wherein the carbohydrate is selected from the group consisting of trehalose and raffinose.

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7. The air dried composition according to claim 4, wherein the neutral chemical compound is betaine.

8. The air dried composition according to claim 4, wherein the organic solvent is DMSO.

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9. The air dried composition according to claim 4, wherein the diol is 1,2-propanediol.

10. The air dried composition according to claim 4, wherein the quaternary ammonium salt is tetramethylammonium chloride.

11. The air dried composition according to claim 4, wherein the anionic polymer is dextran.

12. The air dried composition according to claim 4, wherein the amino acid is L-threonine.

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13. The air dried composition according to claim 4 or 5, wherein the protein is present in a concentration of 3.7 to 37 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

10 14. The air dried composition according to claim 4 or 6, wherein the carbohydrate is present in a concentration of 1 to 300 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

15 15. The air dried composition according to claim 6, wherein trehalose is present in a concentration of 100 to 300 mM, preferably 150 mM, in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

20 16. The air dried composition according to claim 6, wherein raffinose is present in a concentration of 1 to 10 mM, preferably 5 mM, in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

25 17. The air dried composition according to claim 4 or 7, wherein the neutral chemical compound is present in a concentration of 100 to 400 mM, preferably 400 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

30 18. The air dried composition according to claim 4 or 8, wherein the organic solvent is present in 10% (v/v) in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

19. The air dried composition according to claim 4 or 9, wherein the diol is present in a concentration of 100 to 300 mM, preferably 100 to 150 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

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20. The air dried composition according to claim 4 or 10, wherein the quaternary ammonium salt is present in a concentration of 25 to 75 mM, preferably 25 to 50 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

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21. The air dried composition according to claim 4 or 11, wherein if any, the anionic polymer is present in a concentration of 1 to 10 mM, preferably 1 to 5 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

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22. The air dried composition according to claim 4 or 12, wherein if any, the amino acid is present in a concentration of 10 to 40 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

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23. The air dried composition according to claim 4, wherein if any, PEG is present in a concentration of 1 to 10 mM, preferably 1 mM in the composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water prior to drying.

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24. The air dried composition according to any one of claims 1 to 3, wherein the one or more stabilizing molecules are a combination of a protein in a concentration of 3.7 to 37 mM, a carbohydrate in a concentration of 1 to 300 mM, a neutral chemical compound in a concentration of 100 to 400 mM, preferably 400 mM, an organic solvent in a concentration of 10% (v/v), a diol in a concentration of 100 to 300 mM, preferably 100 to 150 mM, a quaternary ammonium salt in a concentration of 25 to 75 mM, preferably 25 to 50 mM, and any one of an anionic polymer in a concentration of 1 to 10 mM, preferably 1 to 5 mM, an

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amino acid in a concentration of 10 to 40 mM and PEG in a concentration of 1 to 10 mM, preferably 1 mM.

25. The air dried composition according to any one of claims 1 to 3, wherein the one or more
5 stabilizing molecules are a combination of BSA in a concentration of 3.7 to 37 mM, trehalose in a concentration of 100 to 300 mM, preferably 150 mM, raffinose in a concentration of 1 to 10 mM, preferably 5 mM, betaine in a concentration of 100 to 400 mM, preferably 400 mM, DMSO in a concentration of 10% (v/v), 1,2-propanediol in a concentration of 100 to 300 mM, preferably 100 to 150 mM, tetramethylammonium chloride
10 in a concentration of 25 to 75 mM, preferably 25 to 50 mM, and any one of dextran in a concentration of 1 to 10 mM, preferably 1 to 5 mM, L-threonine in a concentration of 10 to 40 mM and PEG in a concentration of 1 to 10 mM, preferably 1 mM.

26. The air dried composition according to any one of claims 1 to 3, wherein the one or more
15 stabilizing molecules are a combination of 3.7 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 150 mM 1,2-propanediol, 25 mM tetramethylammonium chloride, 10 mM L-threonine, and 1 mM PEG.

27. The air dried composition according to any one of claims 1 to 3, wherein the one or more
20 stabilizing molecules are a combination of 3.7 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 100 mM 1,2-propanediol, 25 mM tetramethylammonium chloride, 5 mM dextran, and 1 mM PEG.

28. The air dried composition according to any one of claims 1 to 3, wherein the one or more
25 stabilizing molecules are a combination of 37 mM BSA, 150 mM trehalose, 5 mM raffinose, 400 mM betaine, 10% (v/v) DMSO, 150 mM 1,2-propanediol, 50 mM tetramethylammonium chloride, 1 mM dextran, and 40 mM L-threonine.

29. The air dried composition according to any one of claims 1 to 28, which is obtained by
30 air drying a composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air.

30. The air dried composition according to any one of claims 1 to 28, which is obtained by air drying a composition comprising the nucleotides, the polymerase and the one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air for 20 to 40 minutes, preferably 20 to 30 minutes, preferably 30 minutes, at temperature
5 between 50 to 80 °C, preferably at temperature between 55 to 75 °C, more preferably at temperature of 75 °C.

31. The air dried composition according to any one of claims 1 to 28, which is obtained by air drying a composition comprising the nucleotides, the polymerase and the one or more
10 stabilizing molecules in nuclease free water, in a conventional oven with circulating air for 30 minutes at temperature of 75 °C.

32. A method for preparing the air dried composition according to any one of claims 1 to 31, comprising air drying a composition comprising the nucleotides, the polymerase and the
15 one or more stabilizing molecules in nuclease free water, in a conventional oven with circulating air for 20 to 40 minutes, preferably 20 to 30 minutes, preferably 30 minutes, at temperature between 50 to 80 °C, preferably at temperature between 55 to 75 °C, more preferably at temperature of 75 °C.

20 33. A kit for PCR reaction, comprising the air dried composition according to any one of claims 1 to 31.