

MB583MPF32200 HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit

Kit Contents

| Product Code | Reagents provided | MB583MPF32200 |
|--------------|---|---------------|
| | | 10 NO |
| PF1D | 96 Deep-well Plate for Multi Sample | 20 no |
| LA1096A | Magnetic Rod Tip for Insta NX® Mag32 | 40 no |
| DS0015 | Lysis Solution (AL) | 60 ml |
| DS0013 | Proteinase K | 6.5 ml |
| DS0040 | Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5] | 18 ml |
| DS0003 | RNase A (20mg/ml) | 6.5 ml |
| DS1005A | Magnetic Beads | 6.5 ml |

Intended Use

Recommended for isolation of nucleic acid from various samples like human/ animal blood, plasma, serum, saliva, buccal swabs, buffy coat, cells, urine, paraffin embedded tissue, tissue, nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium.

Introduction

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit for Insta NX® Mag32 provides the fastest and easiest way to purify nucleic acid for reliable use in amplification technologies. HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit can be used for isolation of nucleic acid from a wide variety of samples, but the performance may vary depending on the sample type.

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit

This kit carries out efficient extraction of nucleic acid from wide range of samples. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution to inactivate DNases/ RNases and to ensure isolation of intact nucleic acid. The nucleic acid purification procedure comprises of three steps viz. adsorption of nucleic acid to the magnetic beads, removal of residual contaminants and elution of pure nucleic acid. The magnetic beads have a high binding capacity and high-quality nucleic acid is obtained from various species. The nucleic acid obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

Elution

The yield of nucleic acid depends on the sample type and the number of cells in the sample. A single elution step with Elution Solution will provide sufficient nucleic acid to carry out multiple amplification reactions.

Storage

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- **On receipt store RNase A (DS0003): at 2-8°C.**
- **On receipt store Proteinase K (DS0013): at -20°C.**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- 55°C heating block (For Tissue Preparation)
- 56°C Thermal Shaker (For Paraffin Embedded Tissue Preparation)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Ethanol (75%)
- 1X PBS (Product Code: ML116)
- Insta NX® Mag32 (Product Code: LA1096)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- Collection Tube, 2.0ml (Product Code: PW1139)
- Sterile foam Tipped Swab (Product Code: PW1174)
- Trypsin (Product Code: TC598)
- 0.5M EDTA (pH 8.0) (For Urine Preparation)
- Xylene (MB180)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA017)

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.
(For Tissue and Paraffin embedded tissue Preparation)

2. **Thoroughly mix reagents**

Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

3. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research. RNase A (e.g., bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3' end of unpaired C and U residues.

Unit Definition for RNase A

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37°C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit. It is completely free of DNases and proteases. The specific activity is 90 U/mg.

The product as supplied is stable at room temperature (15-25°C).

Specimen Handling and Collection

Collect human/animal cells, tissues, blood sample, serum, plasma in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage.

Incubate cells, tissues, blood sample, serum, plasma samples on ice for complete thaw. Ensure that the samples are at room temperature (15-25°C) before beginning the protocol.

Collect the buccal/gargled sample with the help of a swab and store the samples at 2-8°C for short term storage or -20°C for long term storage. Incubate the samples on ice for complete thaw. Ensure that the samples are at room temperature (15-25°C) before beginning the protocol.

Collect Urine sample in a sterile container (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the sample is at room temperature (15-25°C) before beginning the protocol.

After use, contaminated material must be sterilized by autoclaving before discarding.

Type of Specimens

Clinical samples: human tissue, blood, cells, serum, plasma, saliva, buccal swabs, buffy coat, urine, paraffin embedded tissue, nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium.

Animal samples: blood, serum, plasma, cells

Procedure

Tissue/ Cells

Tissue Preparation

I. Prepare tissue

Weigh a piece of fresh or frozen tissue and mince quickly either by grinding in motor and pestle or by grinding in liquid nitrogen. If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation. Cut the tissue into small pieces as it enables more efficient lysis. Up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a capped 2.0 ml collection tube (not provided) and continue to step II of Tissue Preparation.

NOTE: Tissue can be harvested, by aliquoting in 2.0 ml collection tubes (not provided) and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing nucleic acid.

II. Digest tissue

Add 180µl of Lysis Solution (AL) (DS0015) and 20µl of the Proteinase K (DS0013) to the tissue. Mix by vortexing. Incubate the sample at 55°C until the tissue is completely digested with no particles remaining. Mix by vortexing occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. Vortex briefly after digestion is completed. **This will be your pre- processed sample.**

Cultured Cell Preparation

I. Harvest cells

- **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto 5×10^6 cells for 5 minutes at $300 \times g$ (≈ 1500 rpm). Discard the culture medium and continue with step II of Cultured Cell Preparation.
- **Suspension cell cultures:** Centrifuge upto 5×10^6 cells for 5 minutes at $300 \times g$ [≈ 1500 rpm]. Discard the culture medium completely and continue with step II of Cultured Cell Preparation.

- II. Resuspend the pellet obtained from step I of Cultured Cell Preparation, in capped 2ml centrifuge tube (not provided) add 200 μ l of Resuspension Solution (1X PBS) (ML116) (not provided) and mix thoroughly. If previously frozen, allow the cell pellet to thaw slightly before resuspending. Add 20 μ l Proteinase K (DS0013). **This will be your pre- processed sample.**

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB554Tissue**” program.
3. Place the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

4. Remove the seal from the 96 Deep-well Plate for Multi Sample (PF1D).

NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add 50 μ l of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th and 12th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.
6. Add 180-200 μ l pre- processed sample in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 μ l of RNase A solution (20 mg/ml) (DS0003) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

7. Add 20 μ l Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.
8. Slide the tray in outward direction and after adding the above solutions place the plates on the tray of the machine.

NOTE: 16 samples can be processed in a single 96 Deep-well Plate for Multi Sample (PF1D).

9. Slide the tray of the machine back to its position and close the door of Insta NX[®] Mag32 machine.

10. Click on the **RUN** option on the home screen.

11. After the run is complete, slide the tray of the machine in outward direction. Remove the 96 Deep Well Plate from the position. Slide the tray back to its position and discard the Magnetic Rod Tip for Insta NX[®] Mag32 (LA1096A). Dispense the eluted nucleic acid from column 6 and column 12 to a new

HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Saliva/ Buccal Swab

Buccal Swab Preparation

NOTE: We recommend using Sterile foam Tipped Swab (Product Code: PW1174) (not provided) for collection of sample from inside of cheek to ensure maximum yield

1. Place the buccal swab into a capped 2.0 ml microcentrifuge tube. Add 400µl of 1X PBS (ML116) to the tube.
2. Centrifuge the tube at 13,000 rpm for 2 minutes. Discard the pellet and transfer the supernatant to a new collection tube (not provided). **This will be your pre- processed sample.**

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB554Saliva**” program.
3. Place the Magnetic Rod Tip for Insta NX® Mag32 (LA1096A) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

4. Remove the seal from the 96 Deep-well Plate for Multi Sample (PF1D).

NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add 50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th and 12th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.
6. Add 20µl of Proteinase K (DS0013) and 400µl Saliva sample /pre- processed sample in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20µl of RNase A solution (20 mg/ml) (DS0003) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**
8. Slide the tray in outward direction and after adding the above solutions place the plates on the tray of the machine.

NOTE: 16 samples can be processed in a single 96 Deep-well Plate for Multi Sample (PF1D).

9. Slide the tray of the machine back to its position and close the door of Insta NX® Mag32 machine.
10. Click on the **RUN** option on the home screen.

11. After the run is complete, slide the tray of the machine in outward direction. Remove the 96 Deep Well Plate from the position. Slide the tray back to its position and discard the Magnetic Rod Tip for Insta NX[®] Mag32 (LA1096A). Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Blood/ Serum/ Plasma/ Buffy coat/ Nasopharyngeal/ Oropharyngeal swab

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB554Blood**” program.
3. Place the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

4. Remove the seal from the 96 Deep-well Plate for Multi Sample (PF1D).

NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add 50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th and 12th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.
6. Add 20µl of Proteinase K (DS0013) and 200µl of sample in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

7. Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.
8. Slide the tray in outward direction and after adding the above solutions place the plates on the tray of the machine.

NOTE: 16 samples can be processed in a single 96 Deep-well Plate for Multi Sample (PF1D).

9. Slide the tray of the machine back to its position and close the door of Insta NX[®] Mag32 machine.
10. Click on the **RUN** option on the home screen.
11. After the run is complete, slide the tray of the machine in outward direction. Remove the 96 Deep Well Plate from the position. Slide the tray back to its position and discard the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A). Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Urine

Sample Pre-treatment procedure

- Centrifuge 5 ml of urine sample in 15 ml centrifuge tube at 13,000 rpm for 5 min. Resuspend the pellet in 500 µl of 1X PBS.
- Add 30 µl of 0.5M EDTA (pH 8.0). Mix and centrifuge at 13,000 rpm for 2 min. Discard supernatant.
- Resuspend the pellet with 200 µl of 1X PBS and mix well. **This will be your pre- processed sample.**

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB554Tissue**” program.
3. Place the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

4. Remove the seal from the 96 Deep-well Plate for Multi Sample (PF1D).

NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add 50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th and 12th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.
6. Add 20µl of Proteinase K (DS0013) and 200µl pre- processed sample in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.**
8. Slide the tray in outward direction and after adding the above solutions place the plates on the tray of the machine.

NOTE: 16 samples can be processed in a single 96 Deep-well Plate for Multi Sample (PF1D).

9. Slide the tray of the machine back to its position and close the door of Insta NX[®] Mag32 machine.
10. Click on the **RUN** option on the home screen.
11. After the run is complete, slide the tray of the machine in outward direction. Remove the 96 Deep Well Plate from the position. Slide the tray back to its position and discard the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A). Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Processing of paraffin embedded tissue

- a) Place a small section (not more than 25 mg) of paraffin-embedded tissue into a 1.5 ml microcentrifuge tube (Not provided).

NOTE: The total amount of sections depends on tissue size, type and age of your samples. Weigh the total amount of tissue obtained.

- b) Add 1 ml of xylene. Rock at room temperature for 5 minutes. Centrifuge at maximum speed (≈ 14000 rpm) for 3 minutes.
- c) Remove supernatant by pipetting. Do not remove any of the pellets.
- d) Repeat steps b-c, 2-3 times.
- e) Add 1 ml of ethanol (96-100%). Rock at room temperature for 5 minutes. Centrifuge at maximum speed (≈ 14000 rpm) for 3 minutes.
- f) Remove supernatant by pipetting. Do not remove any of the pellets.
- g) Repeat steps e-f, 2-3 times.
- h) Vacuum-dry (without heat) or air-dry pellet completely.

- I. **Digest tissue**

Add 180 μ l of Lysis Solution (AL) (DS0015) to resuspend the pellet. Mix well and incubate at room temperature (15-25°C) for 30 minutes.

- II. Add 20 μ l of the Proteinase K (DS0013) to the tissue. Mix by vortexing and incubate the sample at 56°C overnight or until the tissue is completely digested with no particle remaining in a Thermal Shaker.
- III. Centrifuge at maximum speed 20,000 x g ($\approx 15,000$ rpm) for 5-10 minutes and collect supernatant in a new collection tube (not provided). **This will be your pre- processed sample.**

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB554Tissue**” program.
3. Place the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

4. Remove the seal from the **96 Deep-well Plate for Multi Sample (PF1D)**.

NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add 50 μ l of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th and 12th column of the **96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32**.

6. Add **200µl pre- processed sample** into **1st well** and **7th well** of the **96 Deep-well Plate for Multi Sample (PF1D)**.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003) in the **1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32**.

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX[®] Mag32.**
8. Slide the tray in outward direction and after adding the above solutions place the plates on the tray of the machine.

NOTE: 16 samples can be processed in a single 96 Deep-well Plate for Multi Sample (PF1D).

9. Slide the tray of the machine back to its position and close the door of Insta NX[®] Mag32 machine.
10. Click on the **RUN** option on the home screen.
11. After the run is complete, slide the tray of the machine in outward direction. Remove the 96 Deep Well Plate from the position. Slide the tray back to its position and discard the Magnetic Rod Tip for Insta NX[®] Mag 32 (LA1096A). Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Storage of the eluate with purified nucleic acid: The eluate contains pure nucleic acid, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of nucleic acid.

Warning

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

The yield of nucleic acid depends upon the type and the volume of starting material used.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR.

Quality Control

Each lot of HiMedia's HiPurA[®] Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Please refer disclaimer Overleaf.

Safety Information

The HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.









Disposal

User must ensure safe disposal by autoclaving and/ or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail to mb@himedialabs.com.

Symbols

| | | | |
|---|-------------------------------|---|----------------------------------|
|  | Manufacturer |  | Do not use if package is damaged |
|  | Batch code |  | Temperature limit |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use |
|  | Use-by date (YYYY-MM) |  | Catalogue number |

Identification No.: PIMB583MPF32200

Rev. No.: 02

Date of Issue: 2025-04

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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