MB554MPF32200 HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32

Kit Contents

Product Code	Reagents provided	MB554MPF32200
		10 NO
PF1D	96 Deep-well Plate for Multi Sample	20 nos
LA1096A	Magnetic Rod Tip for Insta NX® Mag32	40 nos
DS0015	Lysis Solution (AL)	60 ml
DS0013	Proteinase K	7 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	0.5 ml
DS0003	RNase A (20mg/ml)	7 ml
DS1005A	Magnetic Beads	10 ml

Intended Use

Recommended for isolation of genomic DNA from various samples like human/ animal blood, plasma, serum, saliva, buccal swabs, buffy coat, cells and tissue.

Introduction

HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 can be used for isolation of genomic DNA from a wide variety of samples, but the performance may vary depending on the sample type.

HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32

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

Elution

The yield of DNA depends on the sample type and the number of cells in the sample. A single elution with 50µl of Elution Solution will provide sufficient DNA to carry out multiple amplification reactions.

Storage

HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Materials needed but not provided

- 55°C water bath or heating block (For Tissue Preparation)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- 1X PBS (Product Code: ML116)
- Insta NX® Mag32 (Product Code: LA1096)
- Vortex
- Salivol (Product Code: LA1092)
- Polypropylene sealing film (Product Code: PR21)
- Collection Tube, 2.0ml (Product Code: PW1139)
- Shaking water bath
- Sterile foam Tipped Swab (Product Code: PW1174)
- Trypsin (Product Code: TC598)

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.
   (For 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.

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
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. 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 DNA.

II. **Digest tissue**
Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution 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 x 10^6 cells for 5 minutes at 300 x g (≈1500 rpm). Discard the culture medium and continue with step II of Cultured Cell Preparation.
- **Suspension cell cultures:** Centrifuge upto 5 x 10^6 cells for 5 minutes at 300 x 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 µl of Resuspension Solution (1X PBS) (ML116) (not provided) and mix thoroughly. If previously frozen, allow the cell pellet to thaw slightly before resuspending. **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 rods tip 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 20µl RNase A (DS0003) and 180µl **pre- processed sample** in the **1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

6. **Add 30µl Magnetic Beads (DS1005A) in the 4th and the 10th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

7. 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).

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

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

10. 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's tip for Insta NX® Mag32 (LA1096A). Dispense the eluted DNA from column 6 and column 12 to a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided). The eluate contains pure DNA.

    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

Saliva Preparation

I. Collect saliva sample in a sterile container or Salivol (LA1092) (not provided) and proceed with step 5 for sample addition.

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 / gargled sample into a capped 2.0 ml microcentrifuge tube. Add 400 µl of 1X PBS (ML116) to the tube.

2. Centrifuge the tube at 13,000 rpm for 5 minutes. Discard the pellet and transfer the supernatant to a new collection tube (not provided). **This will be your pre- processed buccal 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 rods tip 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 20µl of Proteinase K (DS0013), 20µl RNase A (DS0003), 500µl **saliva/pre- processed buccal sample** in the **1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

6. **Add 30µl Magnetic Beads (DS1005A) in the 4th and the 10th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

7. 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).

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

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

10. 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's tip for Insta NX® Mag32 (LA1096A). Dispense the eluted DNA from column 6 and column 12 to a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided). The eluate contains pure DNA.

    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

1. Switch on the UV light for 10 minutes prior to use.

2. Select **"MB554Blood"** program.

3. Place the magnetic rods tip 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 20µl of Proteinase K (DS0013), 20µl RNase A (DS0003), 200µl **of sample** in the **1st and the 7th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

6. **Add 30µl Magnetic Beads (DS1005A) in the 4th and the 10th column of the 96 Deep-well Plate for Multi Sample (PF1D) for Insta NX® Mag32.**

7. 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).

8. Slide the tray of the machine back to its position and close the door of Insta NX® Mag32 machine.
9. Click on the **RUN** option on the home screen.
10. 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's tip for Insta NX® Mag32 (LA1096A). Dispense the eluted DNA from column 6 and column 12 to a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided). The eluate contains pure DNA.

    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 DNA: The recommended storage temperature for the eluted DNA is -80°C. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA.

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 DNA 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® Multi Sample Pre- filled Plates for Insta NX® Mag32 is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 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.

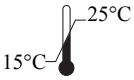
Disposal

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 off in accordance with current laboratory techniques.

Please refer disclaimer Overleaf.

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.



Storage temperature

Do not use if package is damaged

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