MB544MPF32200 HiPurA® Pre- filled Plates for Stool DNA Purification

Kit Contents

Product Code	Reagents provided	MB544MPF32200
		10 NO
PF1G	96 Deep-well Plate for Stool Extraction	20 nos
LA1096A	Magnetic Rod Tip for Insta NX® Mag32	40 nos
DS0015	Lysis Solution (AL)	160 ml
DS0085	Stool Lysis Buffer (SL1)	65 ml
DS0066	Inhibitor Removal Solution (IRSH)	82 ml
DS0013	Proteinase K	6.5 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	1 ml
DS0003	RNase A (20mg/ml)	6.5 ml
DS1005A	Magnetic Beads	6.5 ml

Intended Use

Recommended for isolation of DNA from human and animal stool samples.

Introduction

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

HiPurA® Pre- filled Plates for Stool DNA Purification

This kit carries out efficient extraction of DNA from stool samples. The procedure is optimized for rapid and reliable isolation of high-quality total DNA from upto 250 mg of fresh or frozen stool samples. Stool samples possess typically many compounds that can degrade DNA and inhibit downstream enzymatic procedures. The HiPurA® Pre-filled Plates for Stool DNA Purification contains a unique solution, Inhibitor Removal Solution (IRSH) (DS0066) to remove these inhibitory compounds at an early stage during the extraction process.

The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of DNA. The magnetic beads have a high binding capacity and high-quality DNA is obtained from sample. The purified DNA can directly be used for PCR analysis and other downstream applications.

Elution

The yield of genomic DNA depends on the sample type and the amount of flora present in the sample. A single elution with Elution Solution will provide sufficient nucleic acid to carry out multiple amplification reactions.

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

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Storage

HiPurA® Pre-filled Plates for Stool DNA Purification can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

Materials needed but not provided

- 55°C heating block
- 70°C heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Ethanol (75%)
- Insta NX® Mag32 (Product Code: LA1096)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- Collection Tube, 2.0ml (Product Code: PW1139)
- TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) (Product Code: DS0086)

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.
2. Preheat a water bath or heating block to 70°C.
3. **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.
4. 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 stool 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: Stool sample

Procedure
Sample Pre-treatment procedure-

1. **Resuspension:**
   Take 250 mg of stool sample, add 1ml of TE buffer (DS0086) (not provided). Vortex vigorously and centrifuge at ≥8,000 x g (≥10,000 rpm) for 3 minutes. Discard the supernatant.

2. Resuspend the pellet thoroughly in 500 µl of Lysis Solution (AL) (DS0015). Pipet out 200 µl of the resuspended solution in a new 2ml capped collection tube (not provided) and prepare for lysis.

3. **Lysis**
   To 200 µl of resuspended solution, add 20 µl of the Proteinase K Solution (20 mg/ml). Mix by vortexing and incubate for 30 minutes at 55°C. If residual RNA is not a concern, continue with step 4.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A solution (DS0003), mix, and incubate for 5 minutes at room temperature (15-25°C), then continue with step 4.

4. **Lysis**
   Add 200 µl of Stool Lysis Buffer (SL1) (DS0085), vortex thoroughly (about 15 seconds), and incubate at 70°C for 10 minutes.

   NOTE: A homogeneous mixture is essential for efficient lysis.

5. **Inhibitor removal**
   Add 250 µl of Inhibitor Removal Solution (IRSH) (DS0066), vortex for few seconds and incubate at 4°C for 5 minutes.

6. Centrifuge the tube for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Collect supernatant in a new collection tube (not provided) and discard pellet. **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 **"MB544Stool"** 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 Stool Extraction (PF1G).

   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 **450µl pre- processed sample** in the **1st and the 7th column of the 96 Deep-well Plate for Stool Extraction (PF1G) for Insta NX® Mag32.**

6. **Add 20 µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the 96 Deep-well Plate for Stool Extraction (PF1G) 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 Stool Extraction (PF1G).
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 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 DNA: The eluate contains pure DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, recommended to be stored at -20°C or lower temperature (-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® Pre- filled Plates for Stool DNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Stool DNA Purification 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.

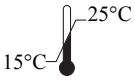
Please refer disclaimer Overleaf.

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.



Storage temperature

Do not use if package is damaged

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