MB544PC16 HiPurA® Pre- filled Cartridges for Stool Nucleic Acid Purification

Kit Contents

Product Code	Reagents provided	MB544PC16
		48PR
PF16D1	Pre- filled Cartridges for Stool nucleic acid purification	48 no
LA1118B	Magnetic Rod Tip	12 no
DS0066	Inhibitor Removal Solution (IRSH)	12.5 ml
DS0003	RNase A (20mg/ml)	1 ml
DS1005A	Magnetic Beads	1 ml
DS0085	Stool Lysis Buffer (SL1)	10 ml
DS0015	Lysis Solution (AL)	25 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	7 ml
DS0013	Proteinase K	1 ml

Intended Use

Recommended for isolation of nucleic acid from human and animal stool samples.

Introduction

HiPurA® Pre- filled Cartridges for Stool Nucleic Acid Purification provides the fastest and easiest way to purify nucleic acid for reliable use in amplification technologies. HiPurA® Pre- filled Cartridges for Stool Nucleic Acid Purification 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 Cartridges for Stool Nucleic Acid Purification

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

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 nucleic acid. The magnetic beads have a high binding capacity and high-quality nucleic acid is obtained from sample. The purified nucleic acid can directly be used for PCR analysis and other downstream applications.

Elution

The yield of nucleic acid 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 Cartridges for Stool nucleic acid Purification can be stored at room temperature (15-25°C) for up to 2 year without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

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

Materials needed but not provided

- 55°C heating block
- 70°C heating block
- Refrigerator at 4°C
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Insta NX® Mag16 (Product Code: LA1118)
- Insta NX® Mag32 (LA1096)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) (Product Code: DS0086)
- Cartridge Holder (Product Code: LA1118CH)

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 (DS0013)**. 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 Cartridges as follows:

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

2. Select **"MB544M16"** program.

3. Open the door of Insta NX® Mag16 machine.

4. Place the Pre-filled Cartridges for Stool Nucleic Acid Purification (PF16D1) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre- filled Cartridges for Stool nucleic acid purification (PF16D1).

   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 **100µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th well of the Pre-filled Cartridges for Stool Nucleic Acid purification (PF16D1).**

6. Add **450µl** of the **pre- processed sample in the 1st well** of the Pre- filled Cartridges for Stool Nucleic Acid Purification **(PF16D1).**

7. **Add 20 µl of Magnetic Beads (DS1005A) in the 1st well of the** Pre- filled Cartridges for Stool Nucleic Acid Purification (PF16D1).

8. After adding the above solutions place the Cartridges along with Cartridge Holder (LA1118CH) on the platform.

9. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

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

10. Close the door of Insta NX® Mag16 machine.

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

12. After the run is complete, remove the cartridge holder along with the cartridges from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from well 6 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure nucleic acid.

    NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

    OR

    Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

***NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip (Pack size- LA1118B-4no/ LA1118B-40no).**

Storage of the eluate with purified nucleic acid: The eluate contains pure nucleic acid. For short-term storage (24-48 hrs) of the nucleic acid, 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 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 Cartridges for Stool nucleic acid Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Cartridges for Stool nucleic acid 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.

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
	Catalogue number		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Contains biological material of animal origin
	Batch code		

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