MB579MPF16 HiPurA® Pre- filled Plate for MTB DNA Purification

Kit Contents

Product Code	Reagents provided	MB579MPF16
		96 PR
PF16N1	Pre-filled Plate for MTB DNA Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS0389	TB Lysis Solution (TL)	20 ml
DS2281	Lysozyme	0.9 g
DS0142	Additive – IV	0.5 ml
DS1005A	Magnetic Beads	2 ml
DS0003	RNase A Solution (20mg/ml)	2 ml
DS0013	Proteinase K	2 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	10 ml

Intended Use

Recommended for isolation of DNA from clinical samples like sputum, body fluid, CSF, Tissue, Pus, biopsy sample, Gastric Lavage, BAL.

Introduction

HiPurA® Pre- filled Plate for MTB DNA Purification provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Plate for MTB DNA Purification 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® Pre- filled Plate for MTB DNA Purification

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 Elution Solution will provide sufficient DNA to carry out multiple amplification reactions.

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

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Storage

Store the HiPurA® Pre- filled Plate for MTB DNA Purification between 15-25°C except certain components as specified on each label. Under recommended condition kit is stable for 2 years.

- **On receipt store Proteinase K (DS0013): at -20°C**
- **On receipt store Additive IV (DS0142): at -20°C**
- **On receipt store RNase A (DS0003): at -2-8°C**
- **On receipt store Lysozyme (DS2281): at 2-8°C**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C**

Materials needed but not provided

- 37°C water bath or heating block
- 55°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Tabletop Microcentrifuge (with rotor for 50.0 ml tubes)
- MB545D- HiPurA® Decontamination Kit for MTB
- Ethanol (96 - 100%)
- Molecular Biology Grade Water (Product code: ML024)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Polypropylene sealing film (Product Code: PR21)

General Preparation Instructions

1. Preheat a water bath or heating block to 37°C.
2. Preheat a water bath or heating block to 55°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 tubes and tips are used for the procedure.
5. Vortex magnetic beads before use.
6. **Prepare Additive Solution (to be freshly prepared)**

a. To 1 ml of TB Lysis Solution (DS0389), add 45 mg Lysozyme powder (MB098) which is provided with the kit. Pipette the mixture up and down or vortex to dissolve the lysozyme.

   NOTE: Lysozyme dissolves readily by pipetting up and down as opposed to vertexing. Excessive vortexing may cause foaming.

b. To 1 ml of above solution, add 25 µl of Additive -IV (DS0142). Mix Properly by gentle pipetting.

   NOTE: For each DNA preparation, 200 µl of Additive Solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at -20°C.

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 sputum sample in 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 sputum 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

Sputum, Body fluids, CSF, Tissue, Pus, Biopsy sample, BAL.

Procedure:

Sr. No.	For Viscous Sputum Sample
1.	Follow protocol A (**Decontamination by NALC Treatment)**

Sr. No.	Body Fluids, PUS, CSF, Non-Viscous Sputum, BAL
1.	Take 500µl of sample, centrifuge at 12000 rpm for 5 minutes, discard the supernatant in a 2ml collection tube (Not Provided)
2.	Proceed with Extraction P**rotocol B (Extraction Protocol)**

Sr. No.	For Tissue
1.	Cut the tissue sample into small pieces in a mortar/Petri plate using a clean pair of forceps, surgical blades and scissors (Not Provided)
2.	Add approximately 2ml of sterile Phosphate buffer (DS0219). Gently invert mix it and continue with **Protocol A (Decontamination by NALC Treatment)**

Protocol A- Decontamination by NALC Treatment (Reagents provided in MB545D kit)

i) Aseptically mix 0.1 g of N-Acetyl-L-cysteine (DS0217) in 20 ml of Sodium citrate-hydroxide buffer (DS0218). Use immediately.

ii) Transfer required volume of specimen to a sterile graduated 50 ml centrifuge tube. Add an equal volume of (N-Acetyl-L-cysteine + Sodium citrate-hydroxide buffer) above solution and mix the contents thoroughly by inverting with the cap tightened.
For Example- In 1ml of specimen add 1ml of (N-Acetyl-L-cysteine + Sodium citrate-hydroxide buffer).

NOTE: Transferring the sample to a centrifuge tube is difficult due to its viscosity. Add the mixture (N-Acetyl-L-cysteine + Sodium citrate-hydroxide buffer) to the vial containing the sample and mix it well.

iii) Vortex the above mixture approximately 20 seconds until the contents are liquefied. Allow the mixture to stand for 15 minutes at room temperature (15-25°C) with occasional gentle shaking by hand. **Do not over process as this will reduce the recovery of *Mycobacterium*.**

iv) Add 3 volumes of Phosphate buffer (DS0219) per 1 volume of sample in the mixture and gently invert the tube. Centrifuge the tube for at least 15 minutes at $\geq$ 3000 x g (5000 rpm) at 4°C.
For example, to 1 mL of sample, add 3 mL of Phosphate Buffer Solution.

v) Carefully decant the supernatant and add 2 ml of phosphate buffer (DS0219), pH 6.8 to the pellet, and resuspend the pellet with the gentle pipetting

vi) Take 1ml of above resuspended solution and centrifuge at 14000 rpm for 3 minutes, discard the supernatant and proceed with pellet for Protocol B.

Protocol B- Extraction Protocol

1. Resuspend the pellet thoroughly in 200µl of Additive Solution **(Prepare Additive Solution as indicated in General Preparation Instructions)** and incubate for 30 minutes at 37°C.
2. **This will be your pre- processed sample.**

Set up processing Plate as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"MB579M16"** program.
4. Remove the seal from the Pre-filled Plate for MTB DNA Purification (PF16N1).

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

5. Add 200µl of **pre- processed sample**, 20µl Proteinase K (DS0013), 20µl RNase A (20 mg/ml) (DS0003) and 20µl of Magnetic Beads (DS1005A) **in the 1st and 7th column of the Pre-filled Plate for MTB DNA Purification (PF16N1).** Place the Plate on the platform.

6. Add **70µl of Elution Buffer (DS0040)** in the **6th and 12th column of the Pre-filled Plate for MTB DNA Purification (PF16N1).**

7. 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.

8. Close the door of Insta NX® Mag16 machine.
9. Click on the **RUN** option on the home screen.

- After the run is complete, remove the Pre-filled Plate for MTB DNA Purification (PF16N1) from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from column 6 and column 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure DNA.

  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.

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® Pre- filled Plate for MTB DNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled for MTB 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.

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.

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.

Please refer disclaimer Overleaf.

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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