MB576MPF16 HiPurA® Pre- filled Plates for Fungal DNA Purification

Kit Contents

Product Code	Reagents provided	MB576MPF16
		96PR
PF16M1	Pre-filled Plates for Fungal DNA Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS0224	HP Fungal Extraction Buffer	90 ml
DS0070	Additive-II	9 ml
DS0071	Additive-III	1.8 g
DS0003	RNase A Solution (20 mg/ml)	2 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	7 ml
DBCA04	HiBead Tubes	96 no
DS1005A	Magnetic Beads	2 ml

Intended Use

Recommended for isolation of DNA from fungal samples.

Introduction

HiPurA® Pre- filled Plates for Fungal DNA Purification provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Plates for Fungal DNA Purification can be used for isolation of DNA from fresh fungal tissue, but the performance may vary depending on the sample type. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure 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.

HiPurA® Pre- filled Plates for Fungal DNA Purification

This kit carries out efficient extraction of DNA from fungus samples. The procedure is optimized for a maximum of 300 mg of wet-weight of the starting material. The sample (fresh) is added to HiBead tubes along with HP Fungal Extraction Buffer. Fungal Extraction Buffer contains CTAB (Cetyltrimethylammonium bromide), a detergent used to break open cells and solubilize the contents. Chlorophyll and some denatured proteins are removed from green Fungal tissue in an organic chloroform- isoamylalcohol step, and the organic phase is separated by centrifugation. Since the extract contains DNA and RNA, RNA can be removed by the addition of RNase A. The flow-through fraction is then mixed with a solution that enhances the binding of DNA to the magnetic particles. The magnetic particles are then carried forward to the washing step to remove trace contaminants. High quality DNA is eluted in the Elution Buffer (ET) provided in the pre-filled plate.

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

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Elution

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

Storage

HiPurA® Pre- filled Plates for Fungal DNA Purification 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 Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- Small mortar and pestle
- Liquid nitrogen (Optional)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) capable of upto ≈13,000 rpm [TabSpin® 012 (LA1090)]
- 65°C water bath or heating block
- Insta NX® Mag16 (Product Code: LA1118)
- Vortex
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Chloroform: Isoamylalcohol (24:1) (Product Code: MB115)

General Preparation Instructions

HP Fungal Extraction Buffer: Immediately prior to use, add 90 µl of Additive-II (DS0070) and 18 mg of Additive-III (DS0071) in 900 µl of HP Fungal Extraction Buffer (DS0224). Preheat the solution to 65°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 260nm when yeast RNA is hydrolyzed at 37^{o}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 Collection and Handling

Collect fungal tissue in a sterile container and store the sample at 2-8°C for short term storage or -20°C for long term storage.

Types of Specimen

Samples: Fungus samples

Protocol

NOTE: Ensure that Additive-II and Additive-III are added to HP Fungal Extraction Buffer (DS0200) as mentioned in General Preparation Instructions.

1. **Lysis**
   To the HiBead Tube (DBCA04) provided, add 900 µl of HP Fungal Extraction Buffer (DS0224) and 300 mg of fungal tissue. Mix by inverting the tube several times or by gentle vertexing.

2. Secure the HiBead tube horizontally on a flat-bed vortex pad using a tape and vortex at maximum speed for 10 minutes.

   NOTE: Ensure that the HiBead tubes rotate freely in the centrifuge without rubbing.

3. Incubate the samples for 30 minutes with occasional inversion at 65°C.

4. Centrifuge the samples at 12,000 rpm for 5 minutes at room temperature (15-25°C).

5. Transfer the supernatant to a new collection tube (not provided) and add 1 ml of Chloroform: Isoamylalcohol (24:1) (not provided) and mix gently by inversion for 5 minutes.

6. Centrifuge the samples at 14,000 rpm for 10 minutes at room temperature (15-25°C).

7. Transfer the top aqueous layer (containing DNA) into a fresh 2.0ml collection tube (not provided) and add 20 µl of RNase A Solution (20 mg/ml) (DS0003). Incubate for 10 minutes at room temperature (15-25°C). **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 **"MB576M16"** program. Open the door of the Insta NX® Mag16 machine.
3. Remove the seal from the Pre-filled Plate for Fungal DNA purification (PF16M1)

   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.

4. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled plate for Fungal DNA Purification (PF16M1).**
5. Add **450µl pre- processed sample** in the **1st and the 7th column of the Pre-filled plate for Fungal DNA Purification (PF16M1).**
6. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plate for Fungal DNA Purification (PF16M1).**
7. Place the Magnetic Rod Tip (LA1118B) onto the machine.

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

   NOTE: 16 samples can be processed in a single Pre-filled Plate for Fungal DNA Purification (PF16M1)

8. Close the door and Click on the **RUN** option on the home screen.

9. After the run is complete discard the Magnetic Rod's Tip (LA1118B). Remove the Pre-filled Plates for Fungal DNA Purification (PF16M1) from the position. Dispense the eluted nucleic acid from column 6 and column 12 to a new Collection Tube, Polypropylene (2.0 ml) (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 cartridge holder along with Cartridges 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 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 Fungal DNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Fungal 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 of 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.

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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