MB568MPF16 HiPurA® Pre- filled Plates for Food Pathogen Purification

Kit Contents

Product Code	Reagents provided	MB568MPF16
		96PR
PF16Q1	Pre-filled Plates for Food Pathogen DNA purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS1005A	Magnetic Beads	2 ml
DS0003	RNase A (20mg/ml)	2 ml
DS0013	Proteinase K	2 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	7 ml
DS0015	Lysis Solution (AL)	49 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	96 no

Intended Use

Recommended for isolation of Food Pathogen.

Introduction

HiPurA® Pre- filled Plates for Food Pathogen Purification provides the fastest and easiest way to purify food pathogen from food samples for reliable applications in PCR, etc. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure pathogen DNA**.**

HiPurA® Pre- filled Plates for Food Pathogen Purification

This kit simplifies isolation of DNA from food samples by the Magnetic Beads based procedure. The food sample is enriched in an enrichment medium (not Provided) as per AOAC guidelines and the cells are harvested by centrifugation. After harvesting, the bacterial cell wall is degraded by Proteinase K. Following lysis, lysate added in Prefilled Plates for food pathogen purification and followed by magnetic beads which promote to selective binding of Nucleic Acid to Magnetic Beads. After the initial binding of Nucleic Acid, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality Nucleic Acid is finally eluted in the Elution Solution provided with the kit.

Elution

The yield of Nucleic Acid depends on the sample type and the number of cells in the sample. A single elution with the 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 Food Pathogen 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 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

- 65°C water bath or heating block (For Lysate Preparation)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) TabSpin® 012 (LA1090)
- Insta NX® Mag16 (LA1118)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Micropipettes and Tips
- Distilled Water
- Autoclave
- Incubator Shaker
- **Selective Enrichment Media for sample preparation as per AOAC guideline:**
  For e.g. Buffered Peptone water (M1494I) for Salmonella & E. CO157:H7: Suspend 20.07 g (the equivalent weight of dehydrated medium per liter) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

General Preparation Instructions

1. Preheat a water bath or heating block to 65°C. (For Lysate 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.
4. Vortex magnetic beads before use.

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).

Enrichment of pathogens:

In order to ensure sensitive detection of pathogens from different variety of food products by PCR, the pathogens need to be enriched in broth.

- Weigh 25g of food sample and add it to autoclaved 225ml of Media.
- Incubate at 37°C for 16-18 hours with shaking at 250 rpm.

Specimen Handling and Collection

For Food

Collect food sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

Type of Specimens

Samples: Food

Procedure

1. **Harvesting of cells**

   Pellet 1.5 ml of an overnight enriched food sample in 2 ml capped collection tube (not provided) by centrifuging for 2 minutes at 13,000 rpm at room temperature (15-25°C). Remove the culture medium and discard.

2. **Resuspend cells**

   Resuspend the pellet thoroughly in 500 µl of Lysis Solution (AL) (DS0015).

3. **Prepare for cell lysis**

   Add 20 µl of the Proteinase K (DS0013) solution to the sample. Mix and incubate for 15-25 minutes at 65°C.

   NOTE: A homogeneous mixture is essential for efficient lysis.

4. **Load sample in HiShredder (DSCA02)**

   Add the entire sample to the HiShredder (DSCA02) placed in a 2.0 ml collection tube (uncapped) and centrifuge for 1 minute at a maximum speed (≈14,000 rpm). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris 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. Open the door of Insta NX® Mag16 machine.
3. Select **"MB568M16"** program.
4. Remove the seal from the Pre-filled Plates for Food Pathogen Purification (PF16Q1).

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 **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Food Pathogen DNA purification (PF16Q1).**
6. Add **200µl of pre- processed sample** into **1st and 7th well of the Pre-filled Plates for Food Pathogen purification (PF16Q1).**

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003) in **1st and 7th well of the Pre-filled Plates for Food Pathogen purification (PF16Q1).**
7. Add **20µl of Magnetic Beads (DS1005A)** in **1st and 7th well of the Pre-filled Plates for Food Pathogen purification (PF16Q1).**
8. 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.
9. Close the door of Insta NX® Mag16 machine.
10. Click on the **RUN** option on the home screen.

- After the run is complete, remove the Pre-filled Plates for Food Pathogen Purification (PF16Q1) from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from column 6 and 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.

Please refer disclaimer Overleaf.

Quality Control

Each lot of HiMedia's HiPurA® Pre- filled Plates for Food Pathogen Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled for Food Pathogen 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.

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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