MB530MPF16 HiPurA® Pre- filled Plates for Paraffin-Embedded Tissue Nucleic Acid Purification

Kit Contents

Product Code	Reagents provided	MB530MPF16
		96 PR
PF16F2	Pre-filled Plates for Paraffin Embedded tissue Nucleic acid	6 No
LA1118B	Magnetic Rod Tip	12 No
DS1637	Digestion Buffer (DB)	20 ml
DS0010A	Binding Solution (BS)	35 ml
DS0013	Proteinase K	2 ml
DS1005A	Magnetic Beads	2 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	7 ml

Intended Use

Recommended for isolation of Nucleic acid from Paraffin-Embedded formalin fixed Tissues.

Introduction

HiPurA® Pre- filled Plates for Paraffin-Embedded tissue Nucleic Acid Purification provides the fastest and easiest way to purify FFPE tissue nucleic acid for reliable use in amplification technologies. The nucleic acid purification procedure using Magnetic beads comprises of three steps viz. adsorption of nucleic acid to the magnetic particles, removal of residual contaminants and elution of pure nucleic acid. HiMedia's Pre-filled Plates allow rapid processing of single as well as multiple samples. The magnetic beads have a high binding capacity and a high-quality nucleic acid is obtained from various species. The Nucleic acid obtained is compatible with downstream applications such as Polymerase chain reaction, sequencing etc.

HiPurA® Pre- filled Plates for Paraffin-Embedded Tissue Nucleic Acid Purification

This kit simplifies isolation of Nucleic acid from formalin-fixed, paraffin embedded tissues such as liver, brain, lung, heart, kidney, spleen, etc. with magnetic bead-based procedure. The tissue is subjected to lysis by Proteinase K in a chaotropic salt solution. Following lysis, the lysate is transferred to pre-filled Plate along with magnetic beads for binding of nucleic acid to the magnetic particles. Two rapid wash steps remove trace salt and protein contaminants resulting in the elution of high-quality nucleic acid in the Elution Buffer (ET).

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

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Elution

The yield of Nucleic acid depends on the sample type and the amount of tissue in the sample. A single elute with the Elution Solution will provide sufficient nucleic acid to carry out multiple amplification reactions.

Storage

HiPurA® Pre- filled Plates for Paraffin-Embedded Tissue Nucleic Acid 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 Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- 50°C heating block to dissolve paraffin
- 56°C heating block or Thermal shaker
- 90°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96-100%)
- Xylene (Product Code: MB180)
- RNase- free pipette tips (aerosol barrier recommended)
- Insta NX® Mag16 (Product Code: LA1118)
- Insta NX® Mag16^{Plus} (Product code: MBLA018)
- Vortex
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

General Preparation Instructions

1. Preheat a heating block or Thermal shaker to 56°C at 600rpm.
2. Preheat a water bath or heating block to 90°C.
3. 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.
5. Vortex magnetic beads before use.

Procedure:

1. **Processing of paraffin embedded tissue:**

a) Place a small section (approx. 2 to 5 section of 10µm depending on the amount of tissue embedded) of the paraffin-embedded tissue into a 2.0 ml microcentrifuge tube (not provided).

NOTE: The total amount of sections depends on tissue size, type and age of your samples. Weigh the total amount of tissue obtained.

b) Add 1 ml of xylene (not provided). Mix well by inversion for 20-30 times. Heat the sample for 3 minutes at 50°C to melt the paraffin. Centrifuge at maximum speed (≈ 14000 rpm) for 3 minutes at room temperature (15-25°C).

c) Discard supernatant by pipetting. Do not remove any of the pellets.

NOTE: If the sample does not form a tight pellet, centrifuge again for 2 minutes. Remove and discard as much solvent as possible without disturbing the pellet.

d) Add 1 ml of ethanol (96-100%) (not provided). Mix well by inversion for 20-30 times. Centrifuge at maximum speed (≈ 14000 rpm) for 3 minutes at room temperature (15-25°C).

e) Discard the supernatant as much as possible without disturbing the pellet.

f) Repeat steps d-e once again.

g) Vacuum-dry (without heat) or air-dry pellet completely.

2. **Digest tissue**
   Add 180µl of Digestion buffer (DB) (DS1637) to resuspend the pellet. Mix well by pulse vortexing at low speed. Add 20µl of the Proteinase K solution (DS0013) to the tissue. Mix by vortexing and incubate the sample at 56°C at 600 rpm for 1hr 30min or longer until the tissue is completely digested with no particle remaining. Mix by vortexing occasionally. Centrifuge briefly to collect any condensation droplets

3. Incubate at 90°C for 1hr in a water bath or a heating block. Centrifuge at maximum speed (≈ 14000 rpm) for 2-3 minutes at room temperature (15-25°C) and transfer the supernatant into a new 2.0ml capped microcentrifuge tube without disturbing the 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 the machine.
3. Select "**MB530M16"** program.
4. Remove the seal from the Pre-filled Plates for Paraffin-Embedded tissue Nucleic Acid (PF16F2).

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

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre- filled Plate for Paraffin-Embedded tissue Nucleic acid (PF16F2).**
6. Before sample addition, add **350µl of Binding Solution (DS0010A) into 1st and 7th column of the Pre-filled Plate for Paraffin-Embedded tissue Nucleic acid (PF16F2).**
7. Add **180-200µl of pre-processed sample and 20µl of Magnetic Beads (DS1005A)** in the **1st and 7th column of the Pre- filled Plate for Paraffin-Embedded tissue Nucleic acid (PF16F2).**
8. After adding the above solutions place the Plates 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 in their place.

10. Close the door of the machine.
11. Click on the **RUN** option on the home screen.
12. After the run is complete, remove the Plates from the position. Discard the Magnetic rod tip (LA1118B). Dispense the eluted nucleic acid from column 6 and 12 to a new Collection Tube (not provided). The eluate contains pure nucleic acid.

    NOTE: A small number of magnetic beads may be observed in the final eluate at the bottom of the tube. Avoid transferring these magnetic beads to your PCR reaction mixture.

Storage of the eluate with purified nucleic acid: The eluate contains pure nucleic acid. For long-term storage, -20°C or lower temperature (-80°C) is recommended. 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 Plates for Paraffin-Embedded tissue Nucleic Acid Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Paraffin-Embedded tissue 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.

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)		
	Batch code		

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