

MB582MPF16 HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification

Kit Contents

Product Code	Reagents provided	MB582MPF16
		96 PR
PF16H	Pre-filled plates for Viral Nucleic Acid	6 No
LA1118B	Magnetic Rod Tip	12 No
DS0013	Proteinase K	2 ml
DS1005A	Magnetic Beads	2 ml
DS0192	Carrier RNA	0.5 mg
DS0042	Elution Solution (RNase free water)	0.55 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	7 ml

Intended Use

Recommended for isolation of HIV, HBV, HCV and other viral nucleic acid.

Introduction

HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification provides the fastest and easiest way to purify viral nucleic acid for reliable use in amplification technologies. HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification can be used for isolation of viral nucleic acid from a wide variety of viruses, but the performance may vary depending on virus type.

HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification

This kit carries out efficient extraction of Viral Nucleic acid from wide range of viral strains. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution to inactivate RNases and to ensure isolation of intact viral nucleic acid. When Carrier RNA is added to Elution Solution (RNase-free Water), it improves the binding of viral nucleic acid to the magnetic beads especially in the case of low-titre samples, and limits possible degradation of the viral nucleic acid due to any residual RNase activity.

Elution

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

Addition of internal control

The internal control allows the user to check both the Nucleic acid purification procedure and possible PCR inhibition.

Purification control:

For this application, internal control should be added in a ratio of 0.1µl per 1µl of elution volume. For example, if the nucleic acid is eluted in 50µl Elution Buffer, 5µl of the internal control should be added.

1. If internal control is added in lysis solution i.e. in Well 1 of Pre-filled cartridge for Viral Nucleic acid. Add 5µl of IC in well 1 of Pre-filled cartridge for Viral nucleic acid. (PF16H)

Refer manufacturer's instructions to determine the optimal concentration of Internal control to be used in purification process. Using a concentration other than that of recommended may reduce amplification efficiency.

Storage

HiPurA® Pre- filled Plates for Viral 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 Carrier RNA (DS0192): at -20°C.**
- **Reconstituted Carrier RNA: -20°C in aliquots to avoid repeated freeze and thaw.**
- **On receipt store Proteinase K (DS0013): at -20°C.**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- RNase- free pipette tips (aerosol barrier recommended)
- Insta NX® Mag16 (LA1118)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non- disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment.
3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours before use. Alternatively, glassware can be treated with DEPC (Diethyl

pyro carbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

5. Solutions (water and other solutions) should be treated with 0.1% DEPC.

General Preparation Instructions

1. 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.

2. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.
3. Vortex magnetic beads before use.

4. Reconstitute Carrier RNA (DS0192)

Carrier RNA (DS0192)	Elution Solution (RNase free water) (DS0042)
0.5 mg	0.5 ml

Dissolve Carrier RNA thoroughly by pipetting. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

Specimen Handling and Collection

Collect Blood, plasma, serum in a sterile container. Thaw the samples on ice before use. Repeated freeze-thaw of samples should be avoided.

Types of Specimen

Clinical samples: Blood, Plasma, serum, nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium.

Procedure:

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 “MB582M16” program.
4. Remove the seal from the Pre-filled plates for Viral Nucleic Acid (PF16H).

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 Viral Nucleic acid (PF16H).
6. Add 20µl of Proteinase K (DS0013), 5µl of Carrier RNA (DS0192), 20µl of Magnetic Beads (DS1005A) & 200µl of sample in the 1st and 7th column of the Pre-filled Plate for Viral Nucleic acid (PF16H).

NOTE: Add internal control in the lysis solution i.e. 1st and 7th column of the Prefilled plate for Viral Nucleic Acid (PF16H) (Refer Addition of internal control).

NOTE: Reconstitute Carrier RNA (Refer General Preparation Instructions)

7. After adding the above solutions place the Plates on the platform.
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.
11. After the run is complete, remove the Pre-filled plate for Viral Nucleic Acid (PF16H) from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 to a new Collection Tube (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.

Storage of the eluate with purified DNA: The eluate contains pure Viral DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended.

Storage of the eluate with purified RNA: The recommended storage temperature for the eluted RNA is -80°C. Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA. Avoid repeated freezing and thawing of the sample which may cause denaturing of Nucleic acid. The Elution Buffer will help to stabilize the Nucleic acid at these temperatures.

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 Viral Nucleic Acid Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Viral 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
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMB582MPF16

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Disclaimer :

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