MB622MPF16 HiPurA® Pre- filled Plates for Water Nucleic Acid Purification

Kit Contents

Product Code	Reagents provided	MB622MPF16
		96PR
PF16L	Pre-filled Plate for Water Nucleic Acid Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS0010	Lysis Solution (C1)	55 ml
ML060	1XTE Buffer (10 mM Tris Cl, 1mM EDTA pH 8.0)	49 ml
DS2281	Lysozyme	0.98 g
DS0192	Carrier RNA	0.6 mg
DS1005A	Magnetic Beads	2 ml
DS0042	Elution Solution (RNase free water)	7 ml
DS0931	Filter Membranes	96 no
DBCA020	Hi-Water Bead Tube	96 no

Intended Use

Recommended for isolation of Nucleic acid from water samples.

Introduction

HiPurA® Pre-filled Plate for Water Nucleic Acid Purification provides the fastest and easiest way to purify Nucleic acid for reliable use in amplification technologies. HiPurA® Pre-filled Plate for Water Nucleic Acid Purification can be used for isolation of Nucleic acid from a wide range of water samples, but performance may vary depending on the sample type.

HiPurA® Pre- filled Plates for Water Nucleic Acid Purification

This kit carries out efficient extraction of Nucleic acid from wide range of water samples. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution (C1) to inactivate RNases and to ensure isolation of intact Nucleic acid. When Carrier RNA is added to Elution Solution (RNase-free Water), it improves the binding of Nucleic acid to the magnetic beads especially in the case of low-titer samples, and limits possible degradation of the viral nucleic acid due to any residual RNase activity.

Elution

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

Storage

HiPurA® Pre- filled Plates for Water 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:

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- **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 Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- RNase- free pipette tips (aerosol barrier recommended)
- Insta NX® Mag16 (Product Code: LA1118)
- Vortex
- Centrifuge (with rotor for 2.0 ml and 15 ml tubes)
- Forceps (Product code: LA710)
- HiPer® Lock Micro- centrifuge tube 2ml- (Product Code- MBLA017)
- Polypropylene sealing film (Product Code: PR21)

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. **Prepare Lysozyme Solution (Product Code: DS2281)**
   Prepare a **20 mg/ml** solution of Lysozyme (approximately 2.115 x 10^6 unit/ml) in TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (ML060). Lysozyme solution should be freshly prepared prior to use.

   Example: In order to make 1ml of Lysozyme solution, dissolve 20mg of lysozyme (provided) in 1ml of 1XTE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (ML060). Pipette the mixture up and down or vortex to dissolve the lysozyme.

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

5. **Reconstitute Carrier RNA (DS0192)**

Carrier RNA (DS0192)	Elution Solution (RNase free water) (DS0042)
0.6 mg	0.6 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 water sample in a sterile container.

Types of Specimen: Water

Procedure:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"MB622M"** program.
4. Remove the seal from the Pre-filled Plate for Water Nucleic acid Purification (PF16L).

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

Sample Pre-processing:

a) Filter 50-1000 ml of water sample through a Filter Membranes (DS0931) to trap the microorganisms or virus particles.

b) Aseptically remove the filter paper using sterile forceps, gently roll the paper and place it inside the Hi-Water Bead tube (DBCA020), such that the top side of the filter paper faces inward.

c) Add 500µl of lysozyme containing TE Buffer **(Refer to General Preparation Instructions)** and mix well by vortexing for 10 minutes.

d) Centrifuge the Hi-Water Bead Tube (DBCA020) at 5000 rpm for 3 minutes (in a 15 ml rotor) at room temperature (15-25°C).

e) Transfer 140µl supernatant to a HiPer® Lock Micro- centrifuge tube 2ml (2.0 ml) (MBLA017) (not provided).

f) Add 560µl of Lysis Solution (C1) (DS0010) and 5.6 µl Carrier RNA (DS0192) to the water sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.

g) Incubate for 10 minutes at room temperature (15-25°C).

h) Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes. Distribute the 350µl lysate into the **1st column** and the remaining 350µl lysate into the **2nd column.** Similarly, **for other samples** distribute the 350µl lysate into **7th column** and the remaining 350µl lysate into **8th column of the Pre-filled Plate for Water Nucleic Acid Purification (PF16L).**

NOTE: Column 1st, 2nd of the Pre- filled Plate for Water Nucleic Acid Purification should contain same sample material. Similarly, Column 7th, 8th of the Pre- filled Plate for Water Nucleic Acid Purification should contain same sample material. Final volume of a single sample is 700µl.

5. Add **50µl of Elution Solution (DS0042)** in the **6th and 12th column** of **the Pre- filled Plates for Water Nucleic Acid Purification (PF16L).**

6. Add 10µl of Magnetic Beads (DS1005A) in the **1st & 2nd and 7th & 8th column of the Pre-filled Plate for Water Nucleic Acid Purification (PF16L).**

   NOTE: 16 samples can be processed in a single Prefilled plate for Water Nucleic Acid purification (PF16L).

7. Place the plate on the platform and close the door of Insta NX® Mag16 machine.

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. Click on the **RUN** option on the home screen.

10. After the run is complete, remove the Pre-filled Plate for Water Nucleic Acid purification from the position. Slide the tray back to its position and discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA1017) (not provided). The eluate contains pure nucleic acid.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

Storage of the eluate with purified nucleic acid: The eluate contains pure nucleic acid, recommended to be stored at lower temperature (-80°C). 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 Water Nucleic Acid Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Water 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

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