MB620A HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter)

Kit Contents

Product Code	Reagents provided	MB620A	
		20 Preps	50 Preps
DS0037	RNA Lysis Solution (HRL)	12.8 ml	32 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml
DS0042	Elution Solution (RNase- Free Water)	3.2 ml	8 ml
DS0192	Carrier RNA	0.2 mg	0.5 mg
ML060	TE Buffer (10 mM Tris Cl, 1mM EDTA pH 8.0)	12 ml	30 ml
MB098	Lysozyme	240 mg	600 mg
DS0931	Filter Membranes	20 nos	50 nos
DBCA020	Hi-Water Bead Tube	20 nos	50 nos
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos

Intended Use

The kit is designed to extract Viral RNA from water samples.

Introduction

HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) can be used for isolation of viral RNA from various water samples, but performance may vary depending on virus type.

HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter)

This kit carries out efficient extraction of viral RNA from wide range of viral strains. Sample is first lysed under the highly denaturing conditions provided by Buffer HRL to inactivate RNases and to ensure isolation of intact viral RNA. When Carrier RNA is added to Elution Solution (RNase-free Water), it improves the binding of viral RNA to the HiElute Miniprep Spin Column especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity.

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 60-80µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reactions.

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Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. The A_{260}-A_{320}/A_{280}-A_{320} ratio should be 1.8 –2.1. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA purified by HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) = 40 x A260 x dilution factor.

Storage

HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. Store the DS0192- Carrier RNA in -20°C temperature on receipt. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

Materials needed but not provided

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 – 100%)

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 equipments.

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 240ºC for four or more hours before use. Alternatively, glassware can be treated with DEPC (Diethyl pyrocarbonate). 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. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.

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

General Preparation Instructions

1. **Prepare Lysozyme Solution (Product Code: MB098)**
   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) **(not provided)**. 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 TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (not provided). 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.

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. **Preparation of Carrier RNA**

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
20	0.2 mg	0.2 ml
50	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.

5. **Preparation of Carrier RNA –Lysis Solution (HRL)**

Number of Preps	Volume of Carrier RNA	Volume of Lysis Solution (HRL)
20	112 µl	11.2 ml
50	280 µl	28 ml

   NOTE: Concentration of Carrier RNA to be used is 10µg/ml

   Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:

   a X 0.56 ml = **b** ml
   b ml X 10 µl/ml = **c** µl

   where, **a** = number of sample to be processed
   b = volume of Lysis Solution (HRL) to be added for '**a**' number of samples
   c = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

6. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	6 ml	18 ml
50	15 ml	45 ml

Specimen Handling and Collection

Collect water sample in a sterile container.

Type of Specimens: Water

Procedure

1. Filter 1000 ml of water sample through a Filter Membranes (DS0931) to trap the microorganisms.
2. 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.
3. Add 500 µl of lysozyme containing TE Buffer and mix well by gentle vortexing for 10 minutes.
4. Centrifuge the Hi-Water Bead Tube (DBCA020) at 5000 rpm for 3 minutes (in a 15 ml rotor).
5. Transfer 140 µl supernatant to a Collection Tube, Polypropylene (2.0 ml) (PW1139).
6. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the cell free sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.
7. Incubate for 10 minutes at room temperature (15-25°C).
8. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.
9. **Binding**
   Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle pipetting.
10. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.
11. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
    Transfer the lysate obtained in the above step onto the HiElute Miniprep Spin Column (DBCA03). Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through after the spin. Repeat step 11 with the remaining sample. Reuse the collection tube.
12. **First Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WS) (DS0012). Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through. Reuse the collection tube.
13. **Second Wash**
    Add another 500 µl of diluted Wash Solution (WS) (DS0012) onto the column. Close the tube gently and centrifuge for 3 minutes at 14,000 rpm to wash the column. Discard the flow-through. Centrifuge for 1 minute at 14,000 rpm to dry the membrane.

14. Transfer the HiElute Miniprep Spin column (Capped) (DBCA03) to a new Collection Tube (Uncapped), Polypropylene (2.0 ml) (DBCA016). Pipet 60-80 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped) (DBCA03). Incubate for 1 minute at room temperature (15-25°C). Close the tube gently and centrifuge for 1 minute at 8,000 rpm.
15. Transfer the eluate to a new Collection Tube, Polypropylene (2.0 ml) (PW1139) for long-term storage.

Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of sample	PCR amplification
Hep A	Observed

References

1. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting

			material (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.
		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another step minute at full speed.
		No DNase treatment	Follow the optional on-column DNase digestion
5.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin Column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

Safety Information

The HiPurA® Water Viral RNA Purification Kit (with Nitrocellulose membrane filter) 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.

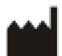
Technical Assistance

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.

Please refer disclaimer Overleaf.

Storage temperature

Do not use if package is damaged



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