

MB622

**HiPurA® Water Nucleic Acid Purification Kit
(with Nitrocellulose membrane filter)**

Kit Contents

Product Code	Reagents provided	MB622	
		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
DS2281	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
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos

Intended Use

The kit is designed to extract Nucleic Acid from water samples.

Introduction

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

HiPurA® Water Nucleic Acid Purification Kit (with Nitrocellulose membrane filter)

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

Elution

The yield of DNA/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 nucleic acid to carry out multiple amplification reactions.

HiElute Miniprep Spin Column (Capped) (DBCA03)

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA/ RNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional nucleic acid isolation techniques. DNA/ RNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit.

Storage

HiPurA® Water Nucleic Acid Purification Kit (with Nitrocellulose membrane filter) can be stored at room temperature (15-25°C) for up to 18 months 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 240°C 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.

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

General Preparation Instructions

1. Prepare Lysozyme Solution (Product Code: DS2281)

Prepare a **20 mg/ml** solution of Lysozyme (approximately 2.115×10^6 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). 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.

- 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:

$$a \times 0.56 \text{ ml} = b \text{ ml}$$

$$b \text{ ml} \times 10 \text{ µl/ml} = c \text{ µl}$$

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 50-1000 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 μ l 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) (DBCA017) 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.

Storage of the eluate with purified DNA: The eluate contains pure 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. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's HiPurA® Water Nucleic Acid 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 Nucleic Acid 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).
		DNA/ RNA still bound to HiElute Miniprep Spin Column	Repeat DNA/ 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 $\geq 8000 \times g$ ($\geq 10,000$ 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.
5.	Nucleic Acid 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 $\geq 8000 \times g$ ($\geq 10,000$ 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 Nucleic Acid 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.

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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Disclaimer :

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HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: techhelp@himedialabs.com Website: www.himedialabs.com