MB615 HiPurA® Viral RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB615		
		20 Preps	50 preps	250 preps
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 ml
DS0192	Carrier RNA	0.28 mg	0.7 mg	3.5 mg
DBCA03	HiElute Miniprep Spin Column (Capped) [in Uncapped Collection Tube]	20 nos	50 nos	250 nos
PW146	Micro Centrifugal Tube-B (1.5ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	20 nos	50 nos	250 nos

Intended Use

Recommended for isolation of Viral RNA from various samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, BAL in Viral Transport Medium and other body fluids.

Introduction

HiPurA® Viral RNA Purification Kit provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Viral RNA can be purified from plasma (treated with anticoagulant EDTA), serum, other body fluids, and infected tissues. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. HiPurA® Viral RNA Purification Kit can be used for isolation of viral RNA from a wide variety of viruses, but performance may vary depending on virus type.

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HiPurA® Viral RNA Purification Kit

This kit carries out efficient extraction of viral RNA from wide range of viral strains like Influenza**,** Dengue, Chikungunya and viral pathogen of animals. 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.
NOTE: For more concentrated RNA lower elution volume (30-40 µl) can be used. Larger elution volumes (up to 100 µl) can also be used but may result in dilution of viral RNA sample.

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 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 RNA isolation techniques. 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® Viral RNA Purification Kit 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 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.
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. **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. **Reconstitute Carrier RNA**

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
20	0.28 mg	280 µl
50	0.7 mg	700 µl
250	3.5 mg	3.5 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.

4. **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
250	1.4 ml	140 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

Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl	Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl
1	0.56	5.6	13	7.28	72.8
2	1.12	11.2	14	7.84	78.4
3	1.68	16.8	15	8.40	84.0
4	2.24	22.4	16	8.96	89.6
5	2.80	28.0	17	9.52	95.2
6	3.36	33.6	18	10.08	100.8
7	3.92	39.2	19	10.64	106.4
8	4.48	44.8	20	11.20	112.0
9	5.04	50.4	21	11.76	117.6
10	5.60	56	22	12.32	123.2
11	6.16	61.6	23	12.88	128.8
12	6.72	67.2	24	13.44	134.4

5. **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
250	75 ml	225 ml

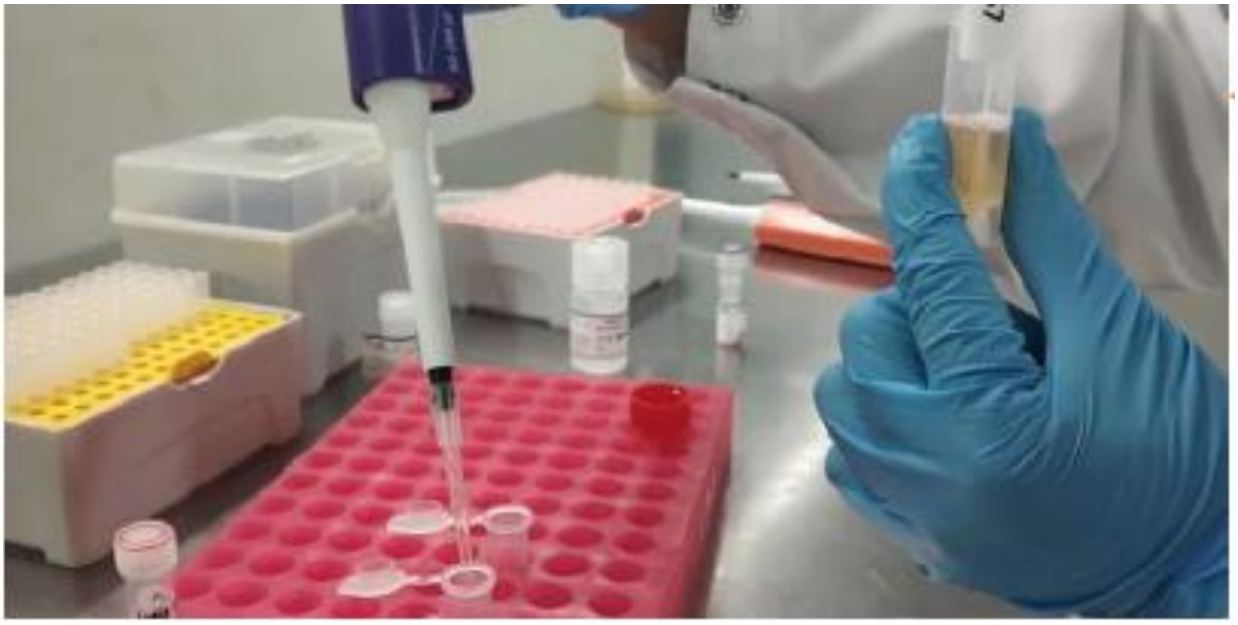
Specimen Handling and Collection

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

Type of Specimens: Clinical samples (Serum, plasma, swabs in viral transport medium and other body fluids)

Procedure

1. Add 140 µl of sample like serum, plasma or body fluid, nasopharyngeal swab, oropharyngeal swab, sputum, BAL, samples collected in Viral Transport medium to Collection Tube, Polypropylene (2.0 ml) (PW1139).
   NOTE: The procedure is optimized for use with 140 µl samples but upto 300 µl sample can be used.
   During work with smaller sample volume (~100 µl) it should be made up to 140 µl with PBS (Phosphate Buffered Saline) before processing. If the initial sample volume is increased the amount of RNA Lysis Solution should be increased proportionally and application of the lysed sample to the HiElute Miniprep Spin Column will require multiple loading steps.

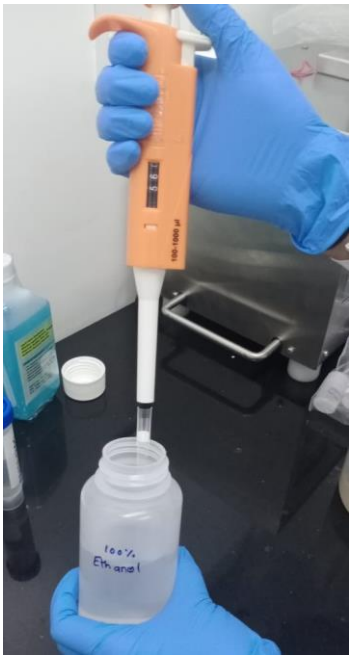


2. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.

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

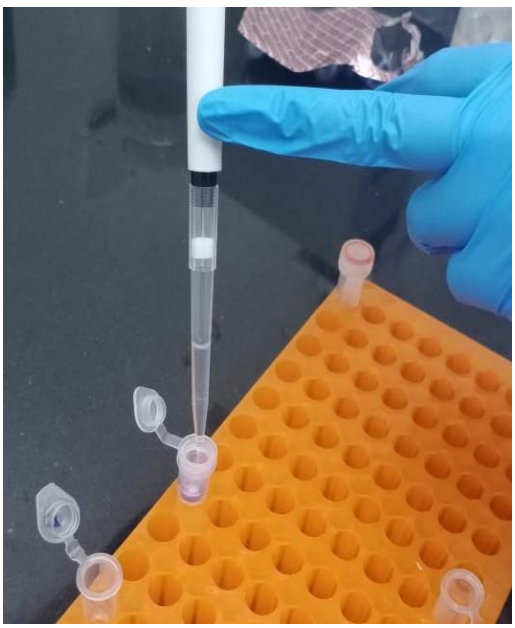
4. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

5. **Binding**
   Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle pipetting.

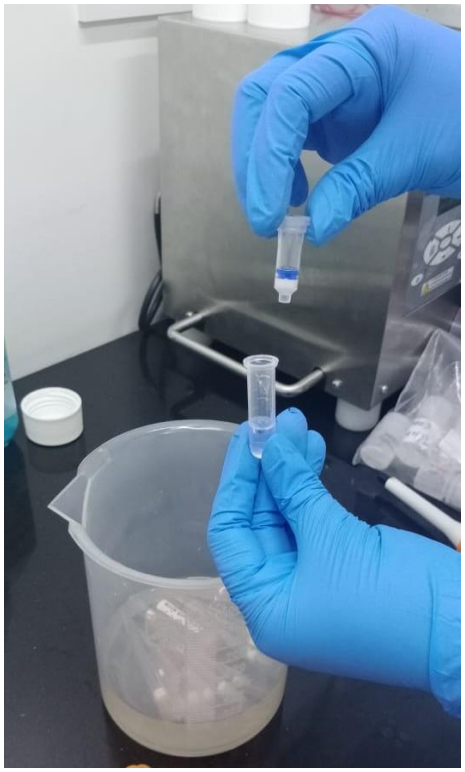


6. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
Transfer the lysate obtained in step 6 onto the HiElute Miniprep Spin Column. Centrifuge at 8,000 rpm for 1 minute.



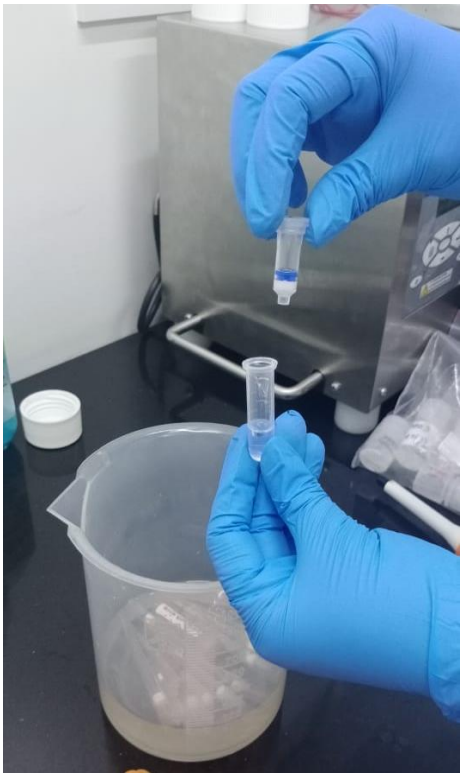
8. Discard the flow-through after the spin. Repeat step 7 with the remaining sample. Reuse the collection tube.



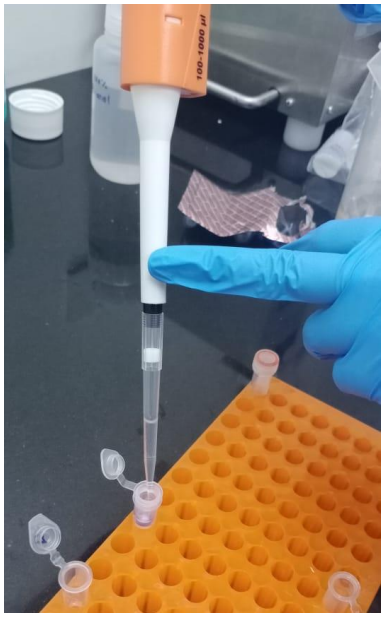
9. **First Wash**
(Prepare Wash Solution as indicated in General Preparation Instructions)
Add 500 µl of diluted Wash Solution (WS) (DS0012). Centrifuge at 8,000 rpm for 1 minute.



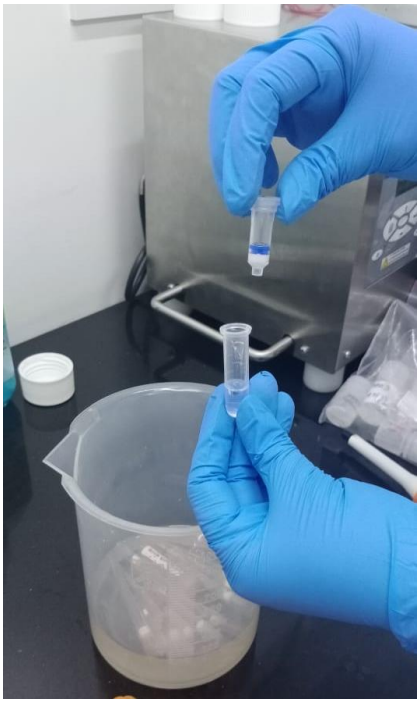
10. Discard the flow-through. Reuse the collection tube.



11. **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.


12. Discard the flow-through. Reuse the collection tube. Centrifuge for 1 minute at 14,000 rpm to dry the membrane.




13. Transfer the HiElute Miniprep Spin column (Capped) (DBCA03) to a Micro Centrifugal Tube 1.5ml (PW146). Pipet 60-80 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Incubate for 1 minutes at room temperature (15-25°C).

14. Close the tube gently and centrifuge for 1 minute at 8,000 rpm. The eluate in the Micro Centrifugal Tube 1.5ml (PW146) contains pure RNA.
NOTE: Place the Micro Centrifugal Tube 1.5ml (PW146) at alternate position in the rotor of the centrifuge machine. The cap of the Micro Centrifugal Tube 1.5ml (PW146) might break if kept side by side in the rotor of centrifuge machine.

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.

Warning

Certified for *in vitro* Diagnostic Use (IVD). 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.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR. Yield of viral RNA isolated from biological samples is usually very less (approx. <1 µg). As a result it is difficult to measure the yield spectrophotometrically. Another point to keep in mind that the carrier RNA will account for most of the RNA present. The yield and efficiency of purification is determined by performing Quantitative RT-PCR. All the QC passed batches have at least 90% recovery of the viral RNA.

Quality Control

Each lot of HiMedia's HiPurA® Viral RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

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
3.	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

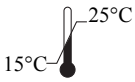
The HiPurA® Viral RNA Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding 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.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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