

MB615M HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)

Kit Contents

Product Code	Reagents provided	MB615M		
		100 Preps	250 preps	480 preps
DS0010	Lysis Solution (C1)	60 ml	150 ml	300 ml
DS0012	Wash Solution Concentrate (WS)	40 ml	100 ml	2X 100 ml
DS0042	Elution Solution (RNase- Free Water)	12 ml	30 ml	60 ml
DS0192	Carrier RNA	1.1 mg	2.75 mg	5.5 mg
DS0013	Proteinase K	2.5 ml	6.25 ml	12 ml
DS1005	Mag Beads	4 ml	10 ml	20 ml

Intended Use

Recommended for isolation of Viral RNA from various samples like nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium and other body fluids.

Introduction

HiPurA® Viral RNA Purification Kit (Magnetic Bead Based) provides the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. HiPurA® Viral RNA Purification Kit (Magnetic Bead Based) can be used for isolation of viral RNA from a wide variety of viruses, but the performance may vary depending on virus type.

HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)

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 Lysis Solution (C1) 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 magnetic beads 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 50µl 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.

Storage

HiPurA® Viral RNA Purification Kit (Magnetic Bead Based) can be stored at room temperature (15-25°C) for up to 2 year 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

- Magnetic separator rack (DS1017)- For Manual Extraction
- RNase- free pipette tips (aerosol barrier recommended)- For Manual, Insta NX® Mag32, Insta NX® Mag96 Extraction
- Ethanol (75%)- For Insta NX® Mag32, Insta NX® Mag96 Extraction
- Ethanol (96-100%)- For Manual Extraction
- Collection Tube, Polypropylene (2.0 ml) (PW1139)- For Manual, Insta NX® Mag32 Extraction
- Insta NX® Mag32 (LA1096)
- Insta NX® Mag96 (LA1097)
- 96 Deep Well Plate for Insta NX® Mag96- For Insta NX® Mag96 Extraction (LA1097B)
- 96 Deep Well Plate for for Insta NX® Mag32- For Insta NX® Mag32 Extraction (LA1096B)
- 96 Elution Plate for Insta NX® Mag96- For Insta NX® Mag96 Extraction (LA1097C)
- Magnetic rod's tip for Insta NX® Mag32- For Insta NX® Mag32 (LA1096A)
- Magnetic tip comb for Insta NX® Mag96- For Insta NX® Mag96 Extraction (LA1097A)
- Vortex
- Polypropylene sealing film (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 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. Vortex magnetic beads before use.

4. Reconstitute Carrier RNA

Number of Preps	Carrier RNA	Elution Solution (RNase free water)
100	1.1 mg	1.1 ml
250	2.75 mg	2.75 ml
480	5.5 mg	5.5 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. Dilute Wash Solution Concentrate (WS) (DS0012) as follows:

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
100	40 ml	120 ml
250	100 ml	300 ml
480	100 ml (in each bottle)	300 ml (in each bottle)

Compatibility with Instruments

This kit is compatible with Instruments like HiMedia- Insta NX® Mag96, HiMedia- Insta NX® Mag32, Thermofisher- KingFisher Flex, Thermfisher – Kingfisher Duo Prime, Genetix- Genetix Purifier HT-96, and MagMax Express-96 Automated DNA/RNA Extraction System.

Specimen Handling and Collection

Collect nasopharyngeal swabs, oropharyngeal swab or nasal aspirate in a sterile container containing appropriate viral transport medium. The specimen can be stored at 4°C upto 48 hours after collection. If any delay is expected, it is recommended to store the specimens at -20°C or lower.

Type of Specimens: Clinical samples (Bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal swab, oropharyngeal swab, nasopharyngeal wash/aspirate or nasal aspirate in viral transport medium and other body fluids)

Instructions before use

- Vortex Mag Beads (DS1005) before use to ensure they are homogenous. Avoid creating bubbles during pipetting.

Procedure (Manual)

1. Add 200 µl of sample to a new Collection Tube (not provided).

NOTE: The procedure is optimized for use with 200 µl sample. During work with smaller sample volume (~25 µl) it should be made up to 400 µl with PBS (Phosphate Buffered Saline) before processing. During work with samples of larger volume, the amount of Lysis Buffer should be increased proportionally.

NOTE: For pooled sample use 400 µl sample volume.

2. Add 530µl of Lysis Solution (C1) (DS0010), 5µl of Proteinase K, 5µl of Carrier RNA and 30 µl of Mag Beads (DS1005) to the sample. Mix by pulse vortexing for 15 seconds.
3. Incubate at room temperature for 10 minutes.
4. **Binding**
Add 300 µl of ethanol (96-100%) (not provided) to the sample, mix well by gentle pipetting and incubate at room temperature (15-25°C) for 5 minutes.
5. Place the tube on magnetic separator rack (DS1017) (not provided) for 1 minute and remove the supernatant by pipetting.

6. **First Wash**

(Prepare Wash Solution as indicated in General Preparation Instructions)

Add 500 µl of diluted Wash Solution (WS) (DS0012). Invert the tube for 3 minutes. Place the tube on magnetic separator rack (DS1017) for 1 minute and remove the supernatant by pipetting.

7. **Second Wash**

Add another 500 μ l of diluted Wash Solution (WS) (DS0012). Invert the tube for 3 minutes. Place the tube on magnetic separator rack (DS1017) for 1 minute and remove the supernatant by pipetting.

8. Dry the sample tubes by placing them at 65°C on heating block for 5 minutes.

NOTE: Keep the lid of the tubes open.

9. Add 50 μ l Elution Solution (RNase-Free Water) (DS0042) to the sample and vortex. Incubate at 65°C on heating block for 5 minutes.

10. Place the tube on magnetic separator rack (DS1017) for 1 minute and remove the supernatant by pipetting to a new collection tube (not provided). The supernatant contains pure RNA.

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.

Procedure for Insta NX[®] Mag32

Accessories for Insta NX[®] Mag32



LA1096A
Magnetic
Rods Tip for
Insta NX[®]
Mag32

LA1096B
96 Deep
Well Plate
for Insta
NX[®] Mag32

Instructions before use

- Vortex Mag Beads (DS1005) before use to ensure they are homogenous.
- The total volume of each well should not exceed more than 1000µl or it may overflow.
- Place a new clean Magnetic rod's tip for Insta NX[®] Mag32 (LA1096A) in the instrument.

NOTE: Always replace the Magnetic rod's tip for Insta NX[®] Mag32 with a new one or it will cause cross contamination of the sample.

- Place the 96 Deep Well Plate for Insta NX[®] Mag32 (LA1096B) containing the sample and reagents into the instrument corresponding to the magnetic rod.
- The procedure is optimized for use with 400 µl sample. During work with smaller sample volume (~25 µl) it should be made up to 400 µl with PBS (Phosphate Buffered Saline) before processing.
Do not process samples more than 400 µl in volume or it may cause spillage on the machine.

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Add reagents to the 96 Deep Well Plate for for Insta NX[®] Mag32 (LA1096B) according to the below table.

Well no.	Sample/ Reagents	Volume (µl)	NOTE
Column 1	Sample	200µl single sample/400 µl for pooled sample	

	Lysis Solution (C1) (DS0010)	530	
	Carrier RNA	5	Reconstitute Carrier RNA (Refer General Preparation Instructions)
	Proteinase K	5	
Column 2	Blank	Blank	
Column 3	Diluted Wash Buffer (DS0012)	600	Prepare Wash Solution as indicated in General Preparation Instructions
Column 4	Ethanol (75%)	600	Not provided
	Mag Beads (DS1005)	30	
Column 5	Ethanol (75%)	600	Not provided
Column 6	Elution Solution (RNase-Free Water) (DS0042)	50	
Column 7	Sample	200µl single sample/400 µl for pooled sample	
	Lysis Solution (C1) (DS0010)	530	
	Carrier RNA	5	Reconstitute Carrier RNA (Refer General Preparation Instructions)
	Proteinase K	5	
Column 8	Blank	Blank	
Column 9	Diluted Wash Buffer (DS0012)	600	Prepare Wash Solution as indicated in General Preparation Instructions
Column 10	Ethanol (75%)	600	Not provided
	Mag Beads (DS1005)	30	
Column 11	Ethanol (75%)	600	Not provided
Column 12	Elution Solution (RNase-Free Water) (DS0042)	50	

3. Fix a new clean Magnetic rod's tip for Insta NX® Mag32 (LA1096A) in the instrument.

NOTE: Always replace the Magnetic rod's tip for Insta NX® Mag32 (LA1096A) with a new one, or it will cause cross- contamination of the sample.

4. Place the filled 96 Deep Well Plate for for Insta NX® Mag32 (LA1096B) onto the machine.
5. Execute the procedure for MB615MA.
6. After the run is complete, slide the platform of the machine. Remove the 96 Deep Well Plate for for Insta NX® Mag32 (LA1096B) from the position. Slide the platform back to its position and discard the Magnetic rod's tip for Insta NX® Mag32 (LA1096A). Dispense the eluted RNA to a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided). The eluate contains pure RNA.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the tube. Avoid transferring these magnetic beads into a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided).

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.

Procedure for Insta NX[®] Mag96

Accessories for Insta NX[®] Mag96



LA1097A
Magnetic
Tip Comb
for Insta
NX[®] MAg96

LA1097B
96-
DeepWell
Plate for
Insta NX[®]
Mag96

LA1097C
96 Elution
Plate for
Insta NX[®]
Mag96

Instructions before use

- Vortex Mag Beads (DS1005) before use to ensure they are homogenous.
- The total volume of each well should not exceed more than 1000µl or it may overflow.
- Place a new clean Magnetic tip comb for Insta NX[®] Mag96 (LA1097A) in the instrument.

NOTE: Always replace the Magnetic tip comb for Insta NX[®] Mag96 (LA1097A) with a new one or it will cause cross contamination of the sample.

- Place the 96 Deep Well Plate for Insta NX[®] Mag96 (LA1097B) and 96 Elution Plate for Insta NX[®] Mag96 (LA1097C) containing the sample and reagents into the instrument according to the plate position mentioned in the table below.
- The procedure is optimized for use with 400 µl sample. During work with smaller sample volume (~25 µl) it should be made up to 400 µl with PBS (Phosphate Buffered Saline) before processing.
Do not process samples more than 400 µl in volume or it may cause spillage on the machine.

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[®] Mag96 machine.
3. Take four blocks of 96 Deep Well Plate for Insta NX[®] Mag96 (LA1097B) and one 96 Elution Plate for Insta NX[®] Mag96 (LA1097C). Add samples and reagents to the 96 well plates according to the below table.

Plate position	Sample/ Reagents	Volume (µl)	NOTE
1	Blank	Blank	
2	Blank	Blank	
3	Sample	200µl single sample/400 µl for pooled sample	
	Lysis Solution (C1) (DS0010)	530	
	Carrier RNA	5	Reconstitute Carrier RNA (Refer General Preparation Instructions)
	Proteinase K	5	
4	Blank	Blank	
5	Diluted Wash Buffer (DS0012)	600	Prepare Wash Solution as indicated in General Preparation Instructions
6	Ethanol (75%)	600	Not provided
	Mag Beads (DS1005)	30	
7	Ethanol (75%)	600	Not provided
	Magnetic rod's tip	-	
8 96 Elution Plate for Insta NX® Mag96 (LA1097C)	Elution Solution (RNase-Free Water) (DS0042)	50	

- Place a new clean Magnetic tip comb for Insta NX® Mag96 (LA1097A) in the 96 Deep Well Plate for Insta NX® Mag96 (LA1097B) (Plate position 7).

NOTE: Always replace the Magnetic tip comb for Insta NX® Mag96 (LA1097A) with a new one, or it will cause cross- contamination of the sample.

- Place the filled 96 Deep Well Plate for Insta NX® Mag96 (LA1097B) and 96 Elution Plate for Insta NX® Mag96 (LA1097C) onto the machine.
- Execute the procedure for MB615MA.
- After the run is complete, discard the Magnetic tip comb for Insta NX® Mag96 (LA1097A). Remove the 96 Deep Well Plate for Insta NX® Mag96 (LA1097B) and 96 Elution Plate for Insta NX® Mag96 from the position. The 96 Elution Plate for Insta NX® Mag96 contains pure eluted RNA. Seal the plate with sealing film (not provided).

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the tube. Avoid transferring these magnetic beads into a new Collection Tube, Polypropylene (2.0 ml) (PW1139) (not provided).

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.

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.

Limitations

The yield of RNA 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. 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 (Magnetic Bead Based) is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Viral RNA Purification Kit (Magnetic Bead Based) 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



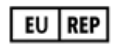








User must ensure safe disposal by autoclaving and/ or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance 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.

Symbols

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

Authorized representative (AR) Address :

	<p>AR Experts B.V. Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands</p>
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Identification No.: PIMB615M

Rev. No.: 07

Date of Issue: 2026-03

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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