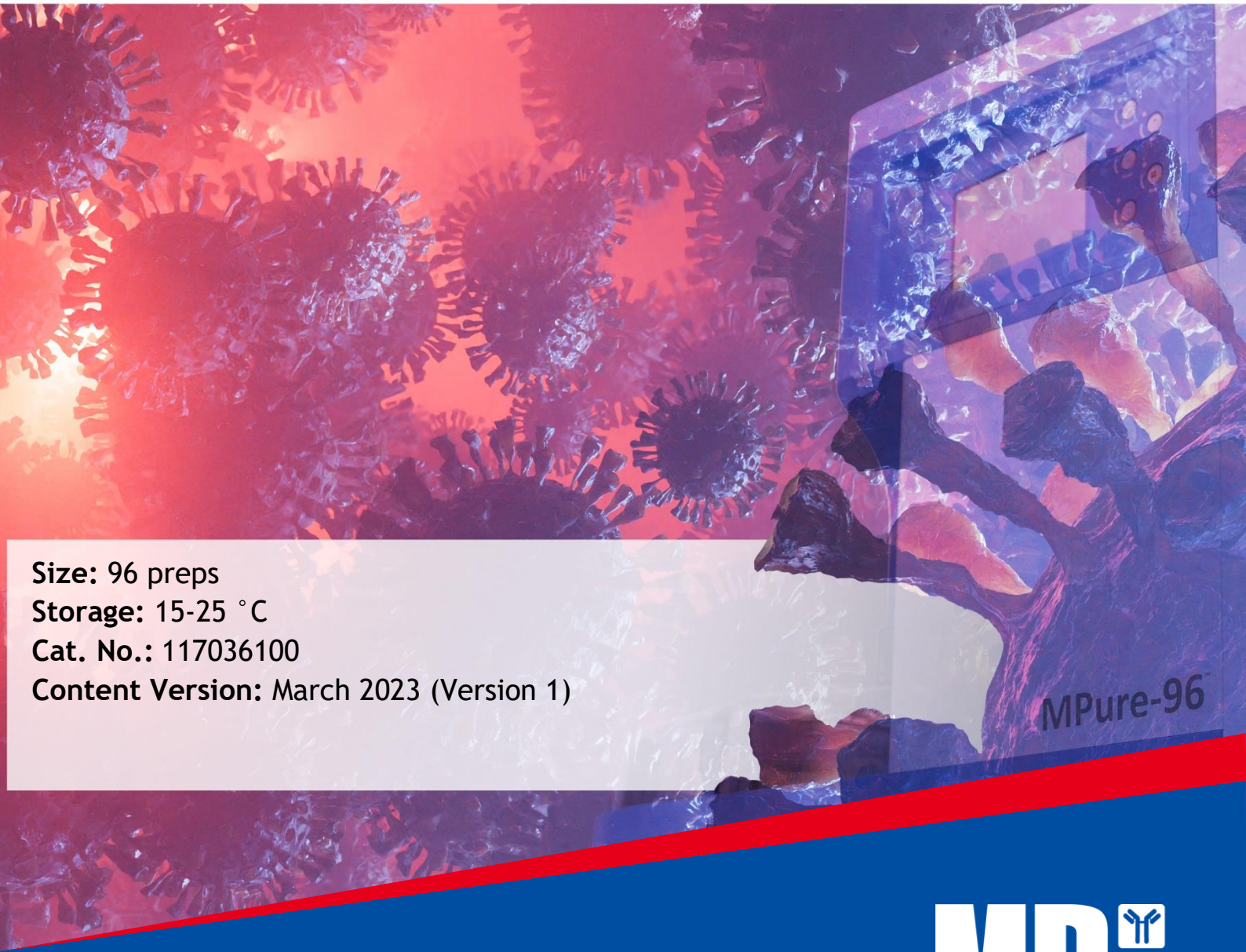
Instruction Manual
Research Use Only

MagBeads

FastRNA Kit for Virus

For Quick and Easy Isolation of Virus RNA
(Ready-to-use for MPure-96™ aNAP System)



Size: 96 preps
Storage: 15-25 °C
Cat. No.: 117036100
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1. Introduction to MagBeads FastRNA Kit for Virus

Extraction of the RNA from viral samples is the most critical step in the detection and diagnosis of viral diseases and its research. Nucleic acid-based identification of pathogens is advantageous compared to conventional immunoassays, in precise identification and to differentiate different strains of closely related pathogenic virus. The genetic information helps in providing the information related to virulence and epidemiology of the analyzed pathogens.

MPs MagBeads FastRNA kit helps to simplify the RNA extraction using a simple protocol containing lysis and RNA purification steps using the Magnetic beads nucleic acid purification technology. This kit is compatible to extract viral RNA from different samples such as cell culture media, water, Phosphate buffer saline and body fluids (Urine, Saliva, serum, nasal swab, oral swab).

The kit components are designed for ready to use for the automated high through put viral RNA extraction using all major automation platforms such as MPure-96, kingfisher, Magna pure, Mgi NE384, Hamilton Nimbus, Tecan and Starlet. The RNA extracted using this kit is of high purity and is compatible for different downstream applications such as Next generation gene sequencing, cDNA synthesis, RT/qPCR and hybridization, etc.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysis Buffer VRM (PF)	30 mL	117035101
RNase-free Water	1 mL	116537003
Carrier RNA	2 x 1 vial	116537054
Proteinase K	2 x 1 vial	116578055
Proteinase K Buffer	3 mL	117035102
Magnetic Beads VRM	1 ea	117036101
Wash Buffer VRM 1	1 ea	117036102
Wash Buffer VRM 2	2 ea	117036103
Elution Buffer	1 ea	117036104
96 spin tips	1 ea	117034111
Quick-start Protocol	1 each	-
Instruction Manual	1 each	-
MSDS & CoA	Available www.mpbio.com	-

2.2 User Supplied Materials

- MPure-96™ aNAP System (Cat No. 07EMC044)
- Single-channel pipettors (5 µL–1000 µL)
- Multichannel pipette (100 µL)
- Nuclease-free, aerosol-preventive tips

3. Storage and Kit Stability

All MagBeads FastRNA Kit for Virus components are stable for at least 12 months from the date of manufacture when stored appropriately at room temperature (15-25°C). Upon receipt, store Carrier RNA and Proteinase K at 2 – 8°C.

Important Consideration Before Use

Please check as appropriate:

- ❑ Prepare Carrier RNA solution and Proteinase K solution (refer to "Things to do before starting" in Section 5).

4. Safety Precautions

Lysis Buffer VRM (PF) and Wash Buffer VRM 1 contain components that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink, or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

5. Protocol

Things to do before starting

- ❑ Prepare Carrier RNA solution:
  Briefly centrifuge the provided vial containing Carrier RNA and resuspend its contents using 350 µL of RNase-free water. Ensure thorough mixing to promote dissolution. Subsequently, divide the carrier RNA solution into smaller aliquots and store them at -20 °C. It is recommended that each aliquot be subjected to no more than three freeze-thaw cycles.
- ❑ Prepare Proteinase K solution:
  Add 1.2 mL of Proteinase K buffer to the Proteinase K vial and ensure proper mixing to facilitate dissolution. Divide the final solution into smaller aliquots and store them at -20 °C for future use.

1. Prepare lysis mixture containing 20 µL Proteinase K Solution, 280 µL **Lysis Buffer VRM (PF)** and 5 µL Carrier RNA Solution per prep, with excess volume.
   Example: For 96 preps, prepare a master mix containing 2 mL Proteinase K Solution, 28 mL Lysis Buffer VRM (PF) and 500 µL Carrier RNA Solution.
2. Add 100 – 200 µL of sample into each well of **Magnetic Beads VRM** plate.
3. Add 305 µL of lysis mixture prepared in step 1 into each well ofMagnetic Beads VRM plate containing the samples.
4. Run program (Virus-RNA) on MPure-96™ aNAP System.
5. At the end of the run, RNA is eluted in **Elution Buffer** plate at **Position 6**.
6. Centrifuge the plate at 5,000g for 5 mins to pellet down residual beads. Alternatively, transfer each eluted RNA from Elution Buffer plate into a clean 1.5 mL microcentrifuge tube and centrifuge at 14,000g for 2 mins. Use clear supernatant for downstream applications.
7. Keep eluted RNA chilled on ice and proceed immediately to perform downstream applications. Store remaining RNA at -80°C and avoid repeated freeze-thawing.

6. Flow Chart

Lysis Mix

Load Sample

Run

Store Elute

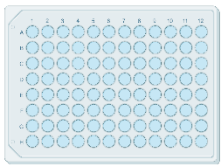
Step 1: Prepare Lysis Mix for N number of preps with excess volume

Up to 96 samples
Note: Each sample will take up the same position in every plate, for example, sample in A1 will go through position A1 in every plate and elute in A1 of Elution Buffer (Plate #6)

	Per prep	96 preps (with excess)
Proteinase K Solution	20 µL	2 mL
Lysis Buffer VRM (PF)	280 µL	28 mL
Carrier RNA Solution	5 µL	500 µL

Step 2: Add Sample and Lysis Mix

Magnetic Beads VRM (Plate #1)



Per well:

100 – 200 µL Sample
305 µL Lysis Mix

Step 3: Load on MPure-96 and run program

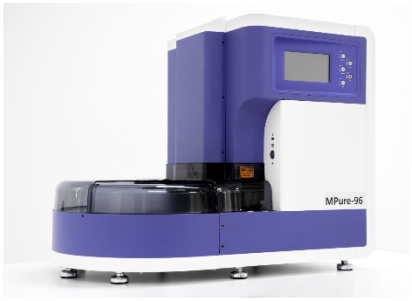
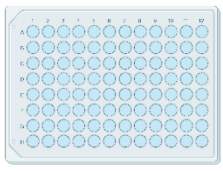


Plate position	1	2	3	4	5	6	7	8
Component	Magnetic Beads VRM	Wash Buffer VRM 1	Wash Buffer VRM 2	Wash Buffer VRM 2	-	Elution Buffer		96 spin tips
Volume (ul)	695	500	500	500	0	100	0	
Preheat	25	25	25			25		
Action	For.U/D	For.U/D	For.U/D	For.U/D	----	For.U/D	----	
Name	LB	WB1	WB2	WB2		EB		TIP

Step	Plate	Temp	Mixing (min)	Spin (rpm)	Collect (sec)	Vapor (min)
1	1		10.0	3000	150	
2	2		3.0	3000	150	
3	3		3.0	3000	150	
4	4		3.0	3000	150	
5	4		0.0	0	0	10.0
6	6	55	5.0	3000	300	
7	0		0.0	0	0	

Step 4: Store Eluted RNA

Elution Buffer (Plate #6)



→ *5min 5K g*

Pellet residual beads

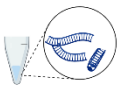
Or

→ Transfer each eluent into a clean 1.5 mL microcentrifuge tube

→ *2min 14K g*

Pellet residual beads

Viral RNA is present in the eluent



Store the eluate at -80°C

7. Data

To determine the sensitivity of the kit. A ten-fold serial dilution of Influenza B virus (ATCC VR-1883DQ), 10^1 copies/µL to 10^5 copies /µL, were spiked into viral transport medium. From each dilution viral RNA was isolated using Magbeads FastRNA Kit for Virus. Equal volume of standard input and RNA extracted from spiked samples were subjected to qPCR amplification. The results were plotted with graph (Figure 1) curve of copy number versus C_t value, demonstrating the kit's ability to isolate RNA from as low as 10 RNA copies in the spiked sample. Result of a control experiment was illustrated in the same graph, where qPCR experiment was conducted using RNA molecules showed overlapping results, indicating the accuracy of the experiment. The isolated RNA was of high purity which is suitable for qPCR further Magbeads FastRNA Kit for Virus was effective in isolating RNA from the sample containing as low as 10 viral particles.

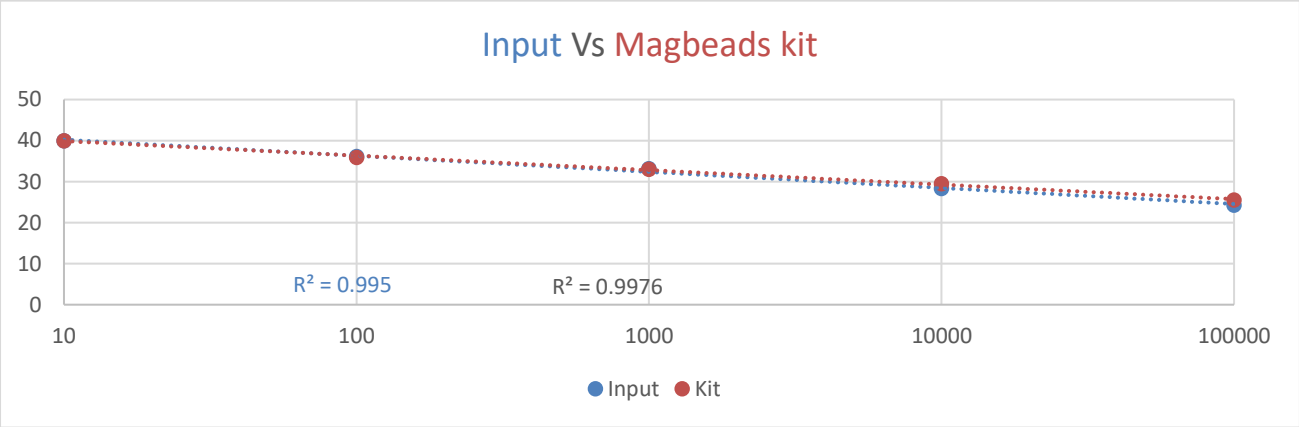


Figure 1: Real time RT-PCR of 10^1 to 10^5 copies of Quantitative Genomic RNA from Influenza B virus (ATCC VR-1883DQ). High RNA recovery is achieved, with almost perfect linearity between Input RNA and RNA extracted using MagBeads FastRNA Kit for Virus.

The virus extraction efficiency of MagBeads FastRNA Kit for Virus also evaluated with various samples. Influenza B virus was spiked into 100 µL and 200 µL of sample and extract using MagBeads FastRNA kit. qPCR was performed to quantify the extracted viral RNA. Consistent Ct value trend were observed for all samples indicating an excellent extraction performance of MagBeads Kit.

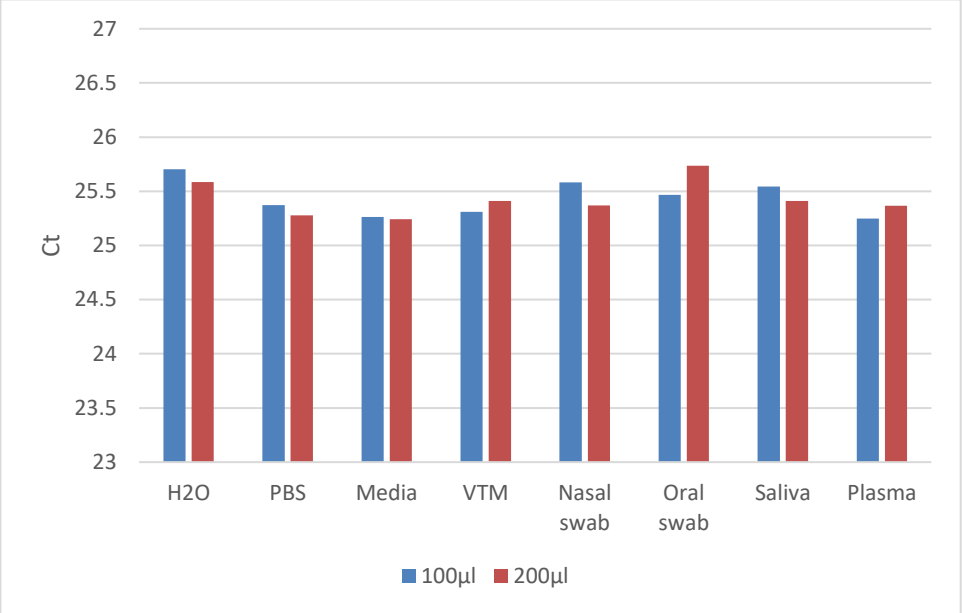


Figure 2: Real time RT-PCR of RNA extracted from various virus-spiked samples. The same amount of Influenza B virus is spiked into 100 µL or 200 µL of the indicated sample, followed by RNA extraction using MagBeads FastRNA Kit for Virus. Similar Ct is obtained for the different samples, indicating that the kit is suitable for virus RNA extraction from cell culture media, swabs and bodily fluids.

8. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**.

Problem	Possible Cause	Recommendation
Little or no RNA recovered	Degradation of Virus RNA in sample	Use fresh samples or freshly frozen samples. Avoid repeated freezing and thawing.
	Carrier RNA not added	Ensure that Carrier RNA solution has been added to the lysis mixture. Lysis mixture should be prepared fresh. Ensure that Carrier RNA solution is prepared and stored appropriately (refer to "Things to do before starting" in section 5).
	Possible RNase contamination	Work with nuclease-free tubes and pipette tips. Handle samples and perform all steps with clean gloves. Decontaminate work surfaces with RNase Erase® (Cat. No. 112440204).
Poor RNA performance for downstream application	Low virus RNA recovery	Refer to Section "Little or no RNA recovered".
	Saturated with carrier RNA	Adjust the amount of carrier RNA added into lysis buffer mixture to determine the optimal carrier RNA concentration suitable for a particular RT-PCR.
	Degradation of eluted RNA	Work with freshly purified RNA and keep RNA chilled on ice after elution. RNA should be stored at -80 °C, freeze thawing should be avoided.

9. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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