

MagBeads

FastRNA Kit for Virus

For Quick and Easy Isolation of Virus RNA
(Ready-to-use on MPure-32™ aNAP System)

Size: 96 preps
Storage: 15-25 °C
Cat. No.: 117035100
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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-32, MGINP-NE32, GENTi advance 32, Bioer GenePure 32, maxwell and tanbeads 4810. 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
Reagent Plate VRM	6 plates	117035103
8 Strips Tip Comb	12 pieces	117033107
Quick-start Protocol	1 each	-
Instruction Manual	1 each	-
MSDS & CoA	Available www.mpbio.com	-

2.2 User Supplied Materials

- MPure-32™ aNAP System (Cat No. 07EMC043)
- Single-channel pipettors (5 µL-1000 µL)
- Nuclease-free, aerosol-preventive tips
- Nuclease-free 1.5 mL microcentrifuge tubes
- Microcentrifuge

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 Reagent Plate VRM 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 μ L of RNase-free water. Be sure to mix it thoroughly. Subsequently, divide the carrier RNA solution into smaller aliquots and store them at $-20\text{ }^{\circ}\text{C}$. It is recommended that each aliquot should not be freeze and thawed more than three times.

❑ Prepare Proteinase K solution:

Add 1.2 mL of Proteinase K buffer into the Proteinase K vial and ensure proper mixing to facilitate dissolution. Divide the final solution into smaller aliquots and store them at $-20\text{ }^{\circ}\text{C}$ for future use.

1. Prepare lysis mixture containing 20 μ L Proteinase K Solution, 280 μ L Lysis Buffer VRM (PF) and 5 μ L Carrier RNA Solution per prep, with excess volume.

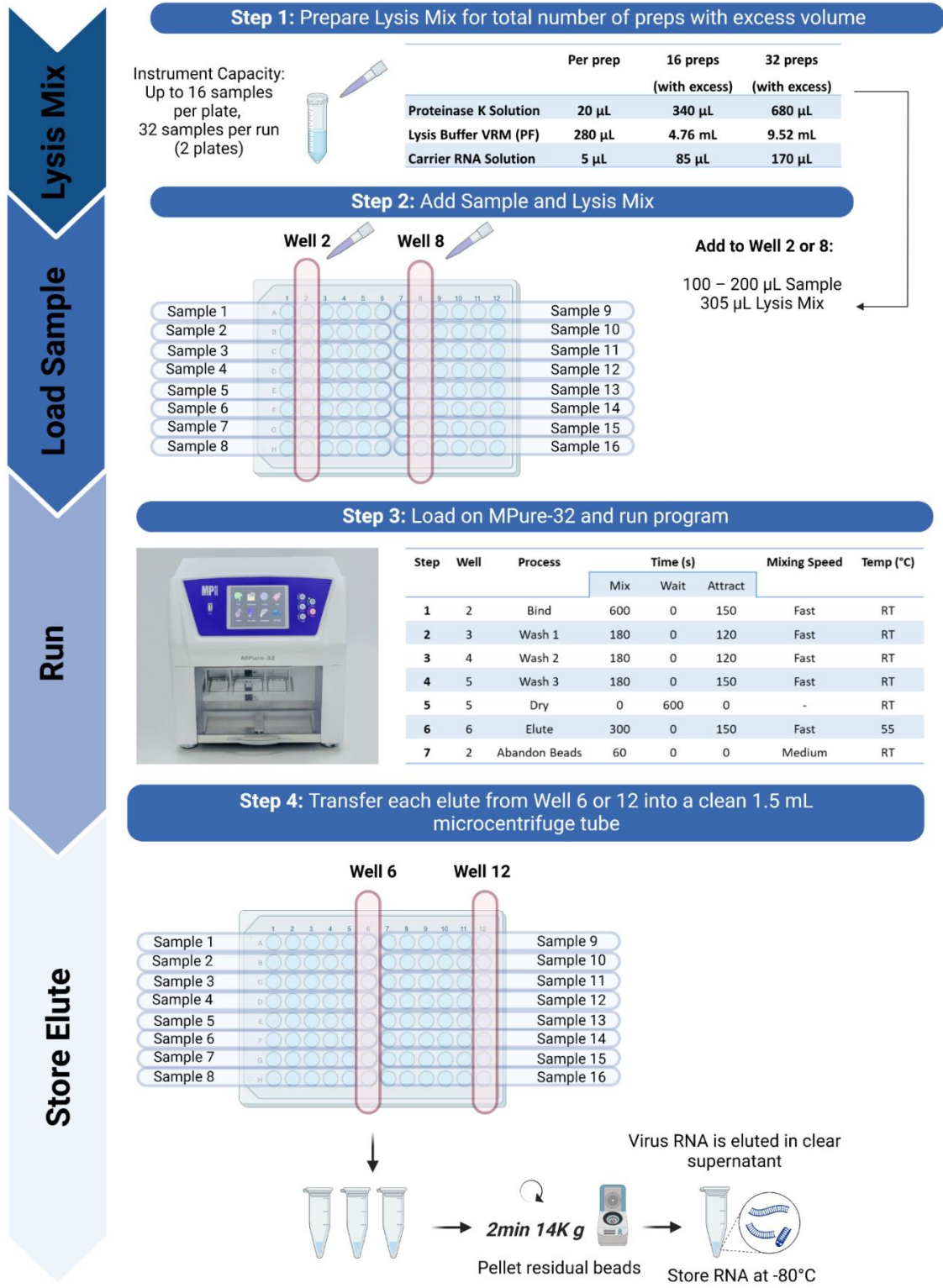
Example: For 16-prep, prepare a master mix containing 340 μ L Proteinase K Solution, 4.76 mL Lysis Buffer VRM (PF) and 85 μ L Carrier RNA Solution.

2. Add 100 - 200 μ L of sample into each well at column position 2 or 8 of the Reagent Plate VRM.
3. Add 305 μ L of lysis mixture prepared in step 1 into each well at column position 2 or 8 containing the samples.
4. Run program (RNA_Virus) on MPure-32™ aNAP System.
5. After the run is completed, place the reagent plate on a magnetic beads separation plate (magnetic base).
6. Transfer the eluted RNA from the wells at column position 6 or 12 into a clean 1.5 mL microcentrifuge tube. Centrifuge the 1.5 mL microcentrifuge tube at 14000x g for 2 mins to pellet down the residual beads if present any. Use clear supernatant for downstream applications.

⚠ Note: RNA is eluted in column position 6 or 12, corresponding to the samples added in the same row in column position 2 or 8 respectively

7. Keep the eluted RNA chilled on ice and proceed immediately to perform the downstream applications. Store the remaining RNA at $-80\text{ }^{\circ}\text{C}$ in aliquots and avoid repeated freeze-thawing cycles.

Flow Chart



6. 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.

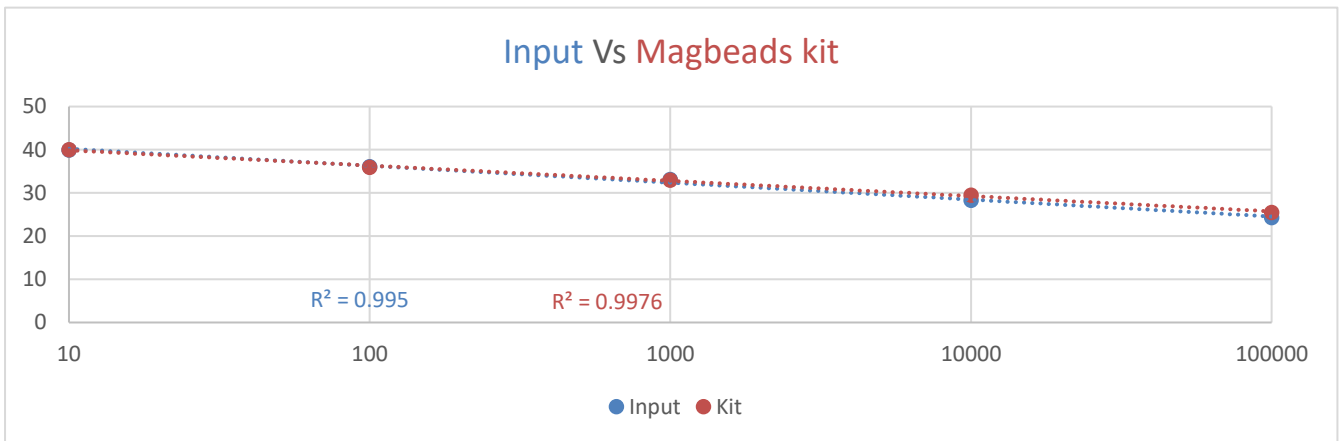


Figure 1: Real time RT-PCR of 10^1 to 10^5 copies of Quantitative Genomic RNA from Influenza B virus (ATCC VR-1883DQ). High RNA recovery is achieved, with almost perfect linearity between Input RNA and RNA extracted using MagBeads FastRNA Kit for Virus.

The virus extraction efficiency of MagBeads FastRNA Kit for Virus also evaluated with various samples. Influenza B virus was spiked into 100 μ L and 200 μ L 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.

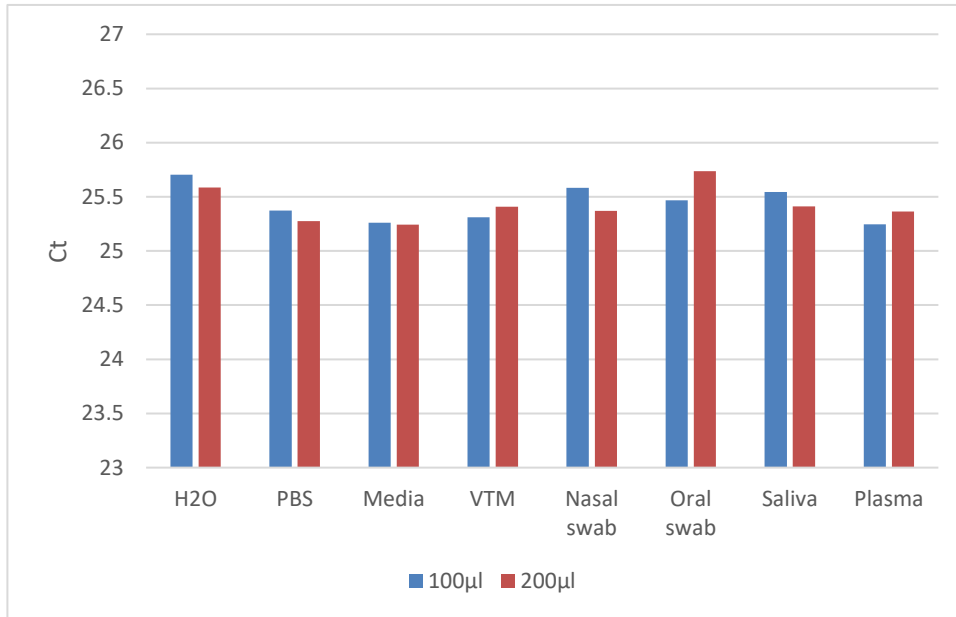


Figure 2: Real time RT-PCR of RNA extracted from various virus-spiked samples. The same amount of Influenza B virus is spiked into 100 μ L or 200 μ L of the indicated sample, followed by RNA extraction using MagBeads FastRNA Kit for Virus. Similar Ct is obtained for the different samples, indicating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

7. 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.

8. 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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Australia

Tel: +61 2.8824.2100
Tel: +61 1800.249.998
Email: custserv.au@mpbio.com

Austria & Germany

Tel: 0800.426.67.337
Tel: 00800.7777.9999
Email: custserv.de@mpbio.com

Belgium

Tel: 00800.7777.9999
Email: custserv.be@mpbio.com

Canada

Tel: +1 800.854.0530
Email: custserv.ca@mpbio.com

China

Tel: +86 400.150.0680
Email: custserv.cn@mpbio.com

Europe

Tel: +33 3.88.67.54.25
Tel: +33 00800.7777.9999
Email: custserv.eur@mpbio.com

France

Tel: +33 3.88.67.54.25
Email: custserv.fr@mpbio.com

India

Tel: +91 22.27636921/22/25
Email: custserv.in@mpbio.com

Italy

Tel: 00800.7777.9999
Email: custserv.it@mpbio.com

Japan

Tel: +81 3.6667.0730
Email: custserv.jp@mpbio.com

Latin America

Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.la@mpbio.com

New Zealand

Tel: +64 9.912.2460
Email: custserv.nz@mpbio.com

North America

Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.na@mpbio.com

Poland

Tel: 00800.7777.9999
Email: custserv.po@mpbio.com

Russia

Tel: +7 495 604.13.44
Email: custserv.rs@mpbio.com

Serbia

Tel: +381 11.242.1972
Email: custserv.se@mpbio.com

Singapore/ APAC

Tel: +65 6775.0008
Tel: +65 6394.7675
Email: custserv.ap@mpbio.com

South Korea

Tel: +82 2.425.5991
Email: custserv.kr@mpbio.com

Switzerland

Tel: 00800.7777.9999
Email: custserv.ch@mpbio.com

The Netherlands

Tel: 00800.7777.9999
Email: custserv.nl@mpbio.com

United Kingdom

Tel: 0800.282.474
Email: custserv.uk@mpbio.com

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