Instruction Manual
Research Use Only

SPINeasy Virus RNA Kit

Spin Column Purification for Easy Isolation of RNA from Virus

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116537050 (50 PREPS)
116537000 (5 PREPS)
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1. Introduction to SPINeasy Virus RNA Kit

SPINeasy Virus RNA Kit is a silica-membrane spin-column kit that enables quick and convenient extraction of virus RNA from cell culture media and bodily fluids such as saliva and serum, without the use of toxic substances such as phenol and chloroform. Through a simple workflow, virus RNA is typically extracted within 20 minutes and is immediately available for downstream applications such as RT-PCR and RT-qPCR.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	Bodily Fluids
Sample amount	100 µL
Elution volume	50 µL
Preparation time	20 min

2. Kit Components and User Supplied Materials

2.1 SPINeasy Virus RNA Kit Component

Components	50 PREPS (Cat.No.: 116537050) Package	Cat. No.
Equilibration Buffer R	12 mL	116547059
Lysis Buffer VR	30 mL	116537051
Wash Buffer VR	6 mL	116537052
RNase-free water	10 mL	116537053
Carrier RNA	1 vial	116537054
Column VR with collection tubes	50 ea	116537055
Quick-Start Protocol	1 ea	-
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- Vortex mixer with adapter
- Microcentrifuge capable of at least 14,000 *g*
- Absolute ethanol (50 mL for preparing Wash Buffer VR and 400 µL per prep for sample preparation)
- Nuclease-free 1.5 mL microcentrifuge tubes
- Optional: 20 mg/mL Proteinase K (only required for extraction from serum samples)
- Optional: 1M DTT (only required for extraction from saliva samples)
- Single-channel pipettors (1 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips
- Biohazard disposal containers
- Microcentrifuge tube rack
- Personal Protective Equipment

3. Storage and Kit Stability

Upon receipt, store Carrier RNA at 2-8˚C. All other components and reagents of the SPINeasy Virus RNA Kit can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer VR and mark the bottle.
- ❑ Prepare Carrier RNA solution according to instructions in Section 6.
- ❑ Prepare two labelled 1.5 mL microcentrifuge tubes per prep: one for sample preparation and another for elution of purified RNA.
- ❑ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 *g* is not feasible.

5. Safety Precautions

Lysis Buffer VR contains a component 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.

6. Protocol

1. **Preparation of Column/Carrier RNA Solution**
   - Add **200 µL Equilibration Buffer** to the **Column** membranes to ensure its performance. Wait at least **1 min** and centrifuge for **30 sec @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Spin down briefly **the vial of carrier RNA** provided and resuspend with **350 µL RNase-free water** (**90 µL** for sample kit). Mix well to dissolve. Aliquot carrier RNA solution and store at **-20 °C**. Do not freeze-thaw each aliquot more than 3 times.

2. **Pre-treatment of Serum Sample**
   - Add **20 µL** 20 mg/mL Proteinase K solution (user supplied) to **100 µL** serum sample and vortex briefly to mix.
   - Incubate for **10 min @ 55°C**.
   - Proceed to perform virus RNA extraction (section 6 No.4).

3. **Pre-treatment of Saliva Sample**
   - Add a final concentration of **5 mM** DTT to saliva sample.
   - Vortex briefly to mix.
   - Proceed to perform virus RNA extraction (section 6 No.4).

4. **Preparation of Carrier RNA-Lysis Buffer mixture**
   - **Carrier RNA-Lysis Buffer** mixture should be prepared fresh. Once prepared, proceed to perform RNA extraction.
   - The following preparation is suitable for a sample volume of **100 µL**. For larger sample volumes, increase the mixture volumes proportionately.
   - For a single prep, add **5 µL Carrier RNA** solution to **300 µL Lysis Buffer VR** and proceed to the extraction protocol.
   - For multiple preps, prepare a master mix of (n + 0.3) times volume of **Carrier RNA-Lysis Buffer** mixture, where n is the number of preps. Refer to Table 1 for recommended volumes of **Lysis Buffer VR** and **Carrier RNA** solution to prepare as master mix for the indicated number of samples.

- Mix by inverting the tube **10 times**. To avoid foaming, do not vortex.

Number of samples (n)	Master mix factor	Lysis Buffer VR volume (mL)	Carrier RNA volume (µL)
1	1	0.3	5
2	2.3	0.69	11.5
3	3.3	0.99	16.5
4	4.3	1.29	21.5
5	5.3	1.59	26.5
6	6.3	1.89	31.5
7	7.3	2.19	36.5
8	8.3	2.49	41.5
9	9.3	2.79	46.5
10	10.3	3.09	51.5
11	11.3	3.39	56.5
12	12.3	3.69	61.5
13	13.3	3.99	66.5
14	14.3	4.29	71.5
15	15.3	4.59	76.5
16	16.3	4.89	81.5
17	17.3	5.19	86.5
18	18.3	5.49	91.5
19	19.3	5.79	96.5
20	20.3	6.09	101.5

Table 1: Preparation of Carrier RNA-Lysis Buffer Master Mix.

RNA Extraction

5. Virus lysis

- Dispense **300 µL Carrier RNA-Lysis Buffer** mix into a nuclease-free 1.5 mL microcentrifuge tube per prep.
- Add up to **100 µL** sample to the **Carrier RNA-Lysis Buffer** mix. If sample volume is

above **100 µL**, increase the volume of carrier RNA-Lysis Buffer mix proportionately and use a larger preparation tube.

- Vortex for **30 sec @ maximum speed**.
- Spin down briefly to collect contents at the bottom of the tube.

6. Bind

- Add **400 µL** absolute ethanol and mix well. If larger volumes of sample and carrier RNA-Lysis Buffer are used, increase the ethanol volume proportionately.
- Transfer up to **800 µL** of the mixture to a **Column VR with collection tube**.
- Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
- If the mixture is more than **800 µL**, repeat until all the mixture has been loaded.

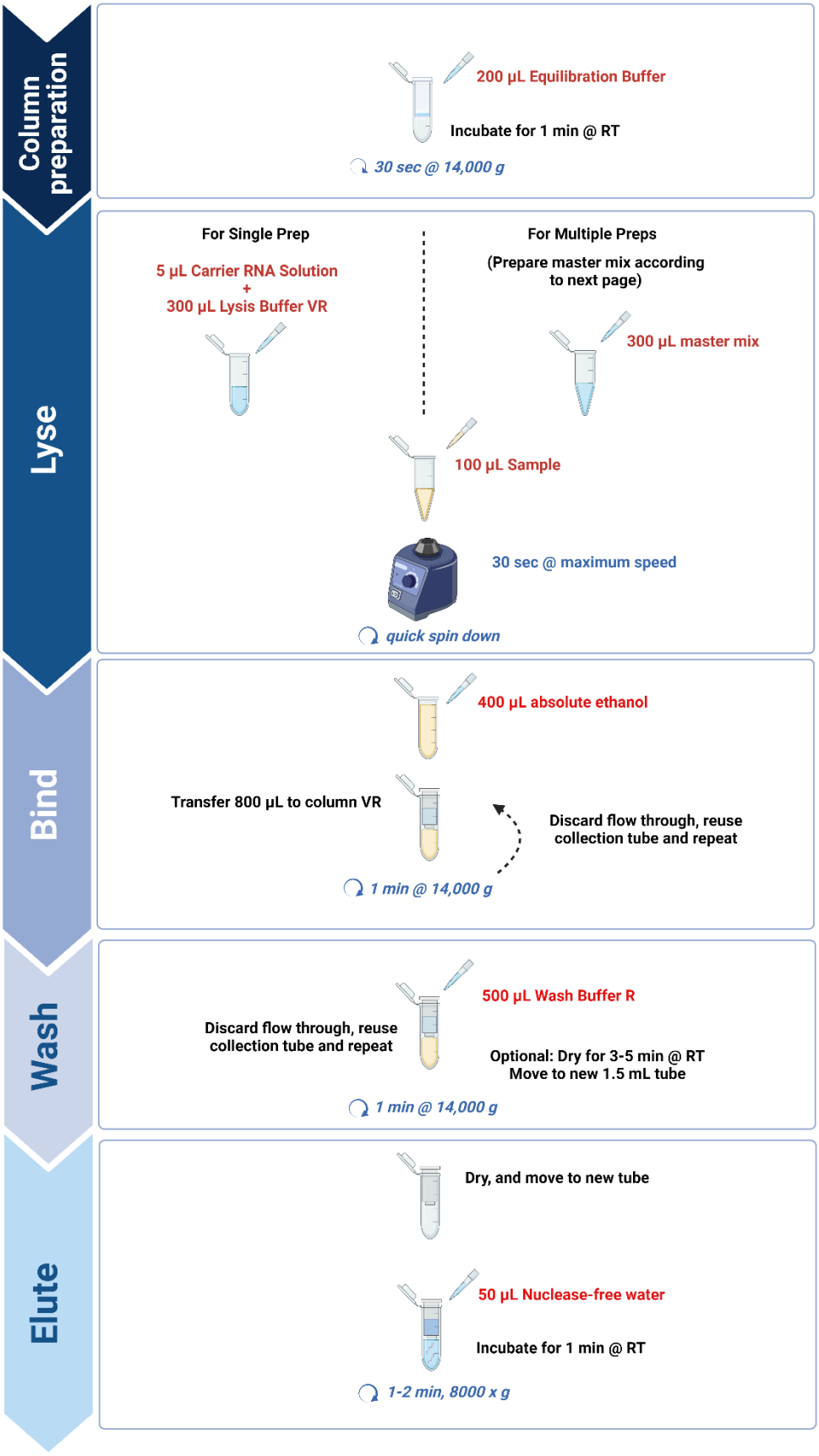
7. Wash

- Add **500 µL Wash Buffer VR** to the column.
- Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
- Add **500 µL Wash Buffer VR** to the column.
- Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
- Centrifuge for an additional **1 min @ 14,000 *g*** to dry column.
- ***Optional:*** *Open the column cap and incubate at room temperature for 3 – 5 mins to dry column completely.*

8. Elution

- Remove collection tube and place column into a clean 1.5 mL microcentrifuge tube.
- Add **50 µL RNase-free water** to the center of the membrane. Incubate for **1 min @ room temperature**.
- Centrifuge for **1 – 2 min @ 8,000 *g*** to elute RNA.
- Eluted RNA will be collected in the microcentrifuge tube.
- Keep eluted RNA samples chilled on ice and proceed to perform downstream application as soon as possible to avoid RNA degradation.
- Store remaining eluted RNA at **-80 °C** in aliquots to avoid repeated freeze-thaw cycles.

7. Flow Chart


Column preparation
200 µL Equilibration Buffer
Incubate for 1 min @ RT
30 sec @ 14,000 g
Lyse
For Single Prep
5 µL Carrier RNA Solution
+
300 µL Lysis Buffer VR
For Multiple Preps
(Prepare master mix according to next page)
300 µL master mix
100 µL Sample
30 sec @ maximum speed
quick spin down
Bind
400 µL absolute ethanol
Transfer 800 µL to column VR
Discard flow through, reuse collection tube and repeat
1 min @ 14,000 g
Wash
500 µL Wash Buffer R
Discard flow through, reuse collection tube and repeat
Optional: Dry for 3-5 min @ RT
Move to new 1.5 mL tube
1 min @ 14,000 g
Elute
Dry, and move to new tube
50 µL Nuclease-free water
Incubate for 1 min @ RT
1-2 min, 8000 x g

8. Data

The following results are RT-PCR of RNA extracted from various virus-spiked samples using SPINeasy Virus RNA Kit.

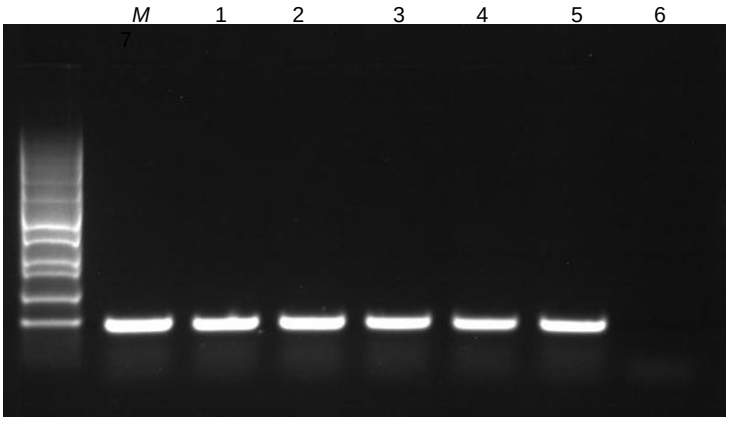


Figure 1: RT-PCR amplification of a viral-specific gene from RNA extracted from the indicated samples spiked with Influenza B virus, using SPINeasy Virus RNA Kit.

M: DNA marker; Lane 1: PBS; Lane 2: DMEM, serum-free; Lane 3: DMEM with 10% FBS; Lane 4: Virus Transport Medium; Lane 5: Saliva; Lane 6: Serum; Lane 7: Negative Control.

9. 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/No RNA Eluted	Inefficient extraction	Ensure the extraction was carried out according to kit manual instructions and the following has been performed correctly: (i) Carrier RNA was added to Lysis Buffer VR; (ii) Ethanol was added to Wash Buffer VR.
	Carrier RNA has degraded.	Once reconstituted, carrier RNA solution should be stored in aliquots at -20 °C. Carrier RNA-Lysis Buffer mixture should be prepared fresh and used immediately.
	Low concentration of virus in the sample.	Concentrate a larger volume of sample using a microconcentrator (not provided).
	Virus RNA has degraded in the sample.	Use fresh samples or samples that have been frozen and thawed only once.
	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).
RNA is not successfully amplified in RT-PCR	Low RNA concentration	Ensure the extraction was carried out according to kit manual instructions and the following has been performed correctly: (i) Carrier RNA was added to Lysis Buffer VR; (ii) Ethanol was added to Wash Buffer VR.
	Too much Carrier RNA.	Adjust the amount of carrier RNA added to Lysis Buffer VR to determine the optimal carrier RNA concentration suitable for the particular RT-PCR.
	Eluted RNA has degraded.	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.

10. 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 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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