SPINeasy™ Virus RNA Kit

Cat. No.: 116537050 (50 PREPS)

Quick-Start Protocol

Revision Oct 2023

Scan QR code for more information from instruction manual

Notes before starting:

- Prepare Carrier RNA Solution: briefly centrifuge the vial of **Carrier RNA** and dissolve in **350 µL RNase-free Water.** Aliquot Carrier RNA solution and store at -20 °C. Do not freeze-thaw aliquot more than 3 times.
- Add **50 mL** absolute ethanol to **Wash Buffer VR** and mark the bottle.
- For saliva and serum samples, refer to back of Quick-Start Protocol for pre-treatment.

Column preparation

1. Pipette **200 µL Equilibration Buffer** into **Column VR with collection tube**. Incubate for **1 min** at room temperature and centrifuge the column for **30 sec @ 14,000 *g*.** Discard flow through and reuse collection tube.
2. Keep the columns aside for later use.

Lyse

3. For a single prep, add **5 µL** Carrier RNA Solution to **300 µL Lysis Buffer VR** and proceed to Step 5 below. For multiple preps, prepare a Carrier RNA–Lysis Buffer master mix using the volumes of Carrier RNA solution and **Lysis Buffer VR** indicated at the back of this protocol.

   Note: *Carrier RNA–Lysis Buffer mixture should be freshly prepared to perform RNA extraction. To avoid foaming, mix by inverting the tube 10 times instead of vortex.*
4. Dispense **300 µL** Carrier RNA–Lysis Buffer master mix into a nuclease-free 1.5 mL microcentrifuge tube per prep.
5. Add up to **100 µL** sample to the Carrier RNA–Lysis Buffer mix. If sample volume is above 100 µL, add **3 volumes** of Carrier RNA–Lysis Buffer mix to **1 volume** of sample and use a larger preparation tube.
6. Vortex for **30 s @ maximum speed** and followed by spinning down briefly to collect contents at the bottom of the tube.

Bind

7. Add **400 µL** absolute ethanol and mix well. If larger volumes of sample and Carrier RNA–Lysis Buffer are used, add equal volume of absolute ethanol to the mixture.
8. Transfer up to **800 µL** mixture to a Column VR with collection tube.
9. Centrifuge for **1 min @ 14,000 *g*.** Discard flow through and reuse collection tube.
10. If the mixture is more than **800 µL**, repeat steps 8 and 9 until all the mixture has been loaded.

Wash

11. Add **500 µL Wash Buffer VR** to the column.
12. Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube. Repeat steps 11 and 12 for second wash.
13. Centrifuge for an additional **1 min @ 14,000 *g*** to dry column.

    Optional: *Open the column cap and air dry at room temperate for **3 - 5 mins.***

Elute

14. Remove collection tube and place column into a clean 1.5 mL microcentrifuge tube.
15. Add **50 µL RNase-free Water** to the center of the membrane. Incubate at room temperature for **1 min.**
16. Centrifuge for **1 – 2 min @ 8,000 *g*** to elute RNA in the microcentrifuge tube.
17. Keep eluted RNA samples chilled on ice and proceed to perform downstream application immediately to prevent degradation. Otherwise, store remaining eluted RNA in -80 °C in aliquots to avoid repeated freeze-thaw cycles.

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Preparation of Carrier RNA–Lysis Buffer Master Mix

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

Pre-treatment of samples:

Pre-treatment of Serum Sample

1. Add **20 µL** 20 mg/mL Proteinase K solution (user supplied) to **100 µL** serum sample and vortex briefly to mix.
2. Incubate at 55 °C for **10 mins**.
3. Proceed to perform virus RNA extraction.

Pre-treatment of Salive Sample

1. Add a final concentration of 5 mM DTT to saliva sample.
2. Vortex briefly to mix.
3. Proceed to perform virus RNA extraction.