ImaSpin® Viral Nucleic Acid Extraction Kit

For purification of Viral Nucleic Acid

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100 %)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IVB Buffer
IVW1 Buffer
IW2 Buffer (Add Ethanol)
RNase-free Water
Proteinase K (Add PK Storage Buffer)
PK Storage Buffer
IV Column
2 ml Collection Tubes

Storage and Stability:

1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4 °C upon arrival.

Description

ImaSpin® Viral Nucleic Acid Extraction Kit is designed by patented technology for purification of Viral Nucleic Acid. The protocol uses buffer contains chaotropic salt to lyse cells and degrade protein. Nucleic Acid will bind to special column. After washing off the contaminants, the purified Nucleic Acid is eluted by low salt elution buffer. Purified Nucleic Acid is suitable for PCR or other enzymatic reactions. The kit is optimised for cell-free samples only.

ImaSpin® Viral Nucleic Acid Extraction Kit Test Data

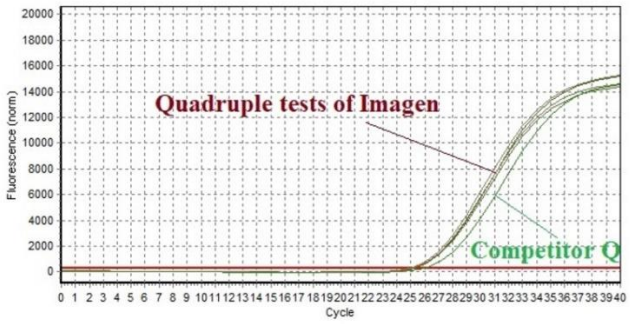

Fig 1. Viral RNA extracted from FCoV (feline coronavirus)

Viral RNA from 140 µl cat ascites samples was extracted using the ImaSpin® Viral Nucleic Acid Extraction Kit and & Competitive brand Q. A Real-time PCR assay was then performed with 4 µl of synthesized cDNA as template, primers, and probe using the TaqMan Real-Time PCR system. The results confirmed that virus RNA can be successfully extracted and the performance of ImaSpin are 1.2-1.5 Ct lower than competitor Q; and the differences of four replicates ImaSpin extraction results are within 0.3 Ct of each other, are extremely stable.

Preparation before using

Add 1.1 ml of PK storage Buffer to the Proteinase K tube and mix by vortexing.
Store prepared Proteinase K (10 mg/ml) at 4 °C.

Protocol

1. Transfer 140 µl of sample (serum, plasma, body fluids, and cell culture supernatant) into a microcentrifuge tube (not provided). If sample volume is less than 140µl, adjust sample volume to 140 µl with PBS (not provided).
2. Add 20 µl of Proteinase K (10 mg/ml) and 560 µl of IVB Buffer, mix well by vortexing.
3. Incubate at 56 °C for 10 minutes.
4. Add 560 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
5. Place an IV Column in a 2 ml Collection Tube.
6. Apply 650 µl of ethanol-added mixture from previous step to the IV Column.
7. Centrifuge a 13,000 rpm (10,000 x g) for 3 minute.
8. Discard the flow-through and apply any remaining mixture from step 4. to the same IV Column.
9. Centrifuge at 13,000 rpm (10,000 x g) for 3 minute and discard the flow-through.
10. Add 600 µl of IVW1 Buffer to the IV Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IV Column back in the 2 ml Collection Tube.
12. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IV Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for1 minute then discard the flow-through. Place the IV Column back in the 2 ml Collection Tube.
14. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
15. Transfer the dried IV Column to a clean 1.5 ml microcentrifuge tube.
16. Add 50 µl of RNase-free Water into the CENTER of the column matrix.
17. Let stand for at least 2 minutes to allow Elution Buffer, RNase-free Water to be completely absorbed.
18. Centrifuge at 13,000 rpm (10,000 x g) for 2 minute to elute the purified Nucleic Acid.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Following ethanol addition, break up any precipitate as much as possible prior to loading to IV Column. ➢ Ensure RNase-free Water is added into the CENTER of the column matrix. ➢ Elute twice to increase yield.