Protocol for the extraction of viral RNA from viral transport medium

1. Add 200 µl of sample into a microtube.

2. Add 400 µl Viral Lysis Buffer. Close the microtube and vortex vigorously for 20 seconds.

3. Incubate at room temperature for 10-15 minutes.

4. Centrifuge at maximum speed for 2 minutes.

5. Transfer the supernatant to a new microtube avoiding touching the pellet that can form.

6. Add **350 µl Ethanol 100%** .Mix well.

7. Transfer half of the lysate to a Spin column with your collection tube. **Centrifuge at 8,000 rpm for 30 seconds.**

8. Pass the other half and centrifuge at 8,000 rpm for 30 seconds.

9. Add **100 µl of RNA Wash Buffer 1.** Centrifuge at maximum speed for 1 minute.

10. Add **700 µl of RNA Wash Buffer 2.** Centrifuge at maximum speed for 1 minute.

11. Centrifuge for **3 minutes** at **maximum speed (11.000 x g)** to completely dry the membrane. Place the column in a 1.5 ml microtube. (not supplied)

12. Elute with 50 µl Elution Buffer or nuclease-free water added to the center of the column membrane. Incubate at room temperature for 2 minutes.
It is very important to add the nuclease-free water in the center of the membrane so that it is completely wetted.

13.Centrifugate at 10.000 rpm for 60 seconds. Collect the 50 µl and re-deposit in the center of the membrane. This increases the performance.

14. Incubate 2 minutes and centrifuge at maximum speed.
If problems are observed in subsequent detections, the volume of the eluate added to the PCR or RT-PCR can be changed.

For any question regarding the work protocols o problems. Please, contact DanaGen-BioTed technical service for any comment or question regarding the protocol info@danagen.es