DANAGENE SPIN VIRAL RNA KIT

REF.0613.1 100 PREPS

1.INTRODUCTION

DANAGENE SPIN Viral RNA Kit is designed for the rapid simultaneous purification **of viral RNA from cell–free samples such as serum, plasma and cerebrospinal fluid.**

Viruses, when lysed by detergent and Proteinase K, release total viral nucleic acids. Then, in the presence of a chaotropic salt, viral nucleic acids bound selectively to glass fiber membrane in a special centrifuge tube. The nucleic acids remain bound while a series of a rapid wash and spin steps removes contaminating cellular components. Finally, low salt elution removes the viral nucleic acids from the glass fiber membrane. The process does not require nucleic acids precipitation, organic solvent extractions, or extensive handling of the nucleic acids.

The DANAGENE Spin Viral RNA Kit can be used for the isolation of viral RNA and DNA from a broad range of RN viruses. **However, performance cannot be guaranteed for every virus species and must be validated by the costumer.**

2. KIT COMPONENTS

	100 Preps	Stock
Viral Lysis Buffer	**45 ml**	Room temperature
Precipitation Protein Buffer	**4 ml**	Room temperature
Wash Buffer 1	**10 ml**	Room temperature
Wash Buffer 2*	**20 ml**	Room temperature
Nuclease-Free water	**8 ml**	Room temperature
Spin Columna RNA	**100 units**	Room temperature
Collection Tubes	**200 units**	Room temperature

(*)These solutions must be prepared as indicated in the section of preliminary preparations.

- Viral Lysis Buffer and Wash Buffer 1* contain guanidine isothiocyanate, which can form reactive components when combined with bleach. Both buffers are irritating agents, for this reason we recommend the use of gloves and glasses for handling. In case of contact with skin or eyes, wash with plenty of water.

2.1 Equipment and additional reagents required

- Ethanol 100 %
- Microcentrifuge.
- Microtubes of 1.5 ml.

2.2 Storage Conditions

All kit components can be stored at indicate temperatures and are stable for at least one year.

3. PROTOCOL

3.1 Preliminary Preparations

- **Add 80 ml of Ethanol 100 %** to the Wash Virus Buffer 2. Keep the container closed to avoid the ethanol evaporation.

3.2 General Remarks

Samples

• Plasma / serum: Avoid working with samples that show hemolysis. After obtaining the plasma or serum, it is important to centrifuge the sample to obtain a cell-free initial material.

• For solid samples such as tissues (5-10 mg) it can be homogenized in 300-400 µl of PBS using a hand-held electric homogenizer or ball-based homogenization systems. Centrifuge the sample and use 200 µl of the particle-free transparent supernatant.

• For feces prepare a suspension with PBS, 10% (w / v) and use the best system to be able to lyse all the viral particles. Centrifuge the sample and use 200 µl of the particle-free transparent supernatant.

• Swabs: Incubate the swab in an adequate amount of buffer (eg PBS) or cell-free medium for 30 minutes with movement. Remove the swab and proceed with 200 µl of the particle-free transparent supernatant.

3.3 Protocol for the extraction of viral RNA from serum, plasma and biological fluids

1. Add 200 µl of sample into a microtube. If you process samples of <200 µl, adjust the final volume to 200 µl using PBS or nuclease-free water.

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.

The incubation time and temperature is critical for lysis as well as for RNA stability. Incubation at room temperature is usually sufficient without significant loss of sensitivity. It is recommended to optimize these points for the type of sample that will be used. Protocols can be compared with and without the use of proteinase K, as well as different incubation times and temperatures.

If the sample is very viscous (sputum), the use of proteinase K or incubation at 70ºC is recommended.

4. Add **30 µl of Precipitation Proteib Buffer.** Vortex and incubate for 1 minute.

5. Centrifuge at maximum speed for 3 minutes.

6. Transfer the supernatant to a new microtube.

7. Add **350 µl Etanol 100%** .Mix well.

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

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

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

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

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

13. Elute with 50 µl Elution Buffer or nuclease-free water added to the center of the column membrane.

It is very important to add the nuclease-free water in the center of the membrane so that it is completely wetted.

14. Incubate at room temperature for 2 minutes.

15.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.

16. 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.

4. TOUBLESHOOTING

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