DANAGENE SPIN VIRAL DNA/RNA KIT

REF.0612.1 100 PREPS

1.INTRODUCTION

DANAGENE SPIN Viral DNA/RNA Kit is designed for the rapid simultaneous purification **of viral DNA and 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 DNA/RNA Kit can be used for the isolation of viral RNA and DNA from a broad range of RNA and DNA viruses. **However, performance cannot be guaranteed for every virus species and must be validated by the costumer.**

2. KIT COMPONENTS

Product		Ta
Viral Lysis Buffer	25 ml	RT
Wash Virus Buffer 1*	36 ml	RT
Wash Virus Buffer 2 *	20 ml	RT
Elution Buffer	10 ml	RT
Proteinase K*	105 mg	-20ºC
Carrier RNA*	1 mg	-20ºC
Spin Columns	100 unid	RT
Collection Tubes	200 unid	RT

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

- Lysis Viral Buffer and Wash Virus Buffer 1* contain guanidine hydrochloride, 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

- Dissolve the proteinase K in **5.2 ml of nuclease-free water** and store at –20ºC. It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.

    - **Add 20 ml of Ethanol 100 %** to the Wash Virus Buffer 1. Keep the container closed to avoid the ethanol evaporation.

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

    - **Carrier RNA:** Increases the binding of viral nucleic acids to the glass fiber membrane and reduces the risk of viral RNA degradation. The eluates obtained with this kit contain both viral RNA and carrier RNA, **and the amounts of carrier RNA can exceed the amounts of viral RNA.** The calculations of how much eluate should be added to subsequent applications should be based on the amounts of carrier RNA added.

To obtain high levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to the Viral Lysis Buffer. If you want to use less carrier RNA per sample, you need to validate the amount of carrier RNA needed for each sample type and subsequent applications.

3.2 Protocol for preparing of carrier RNA

a) Add 500 µl of Elution Buffer to the microtube containing the carrier RNA supplied in a microtube with the kit for obtaining a stock solution.
b) Mix and aliquot in 25-50 µl, store at -20ºC. Avoid repeated heating-cooling cycles.
c) Calculate the volume of Viral Lysis Buffer / carrier RNA mixture required to process the required number of samples simultaneously. For example, add 50 µl of carrier to 2.5 ml of Viral Lysis buffer
d) In a sterile microtube, add the volume of carrier RNA stock solution to the volume of Viral Lysis Buffer. Mix gently with micropipette, up and down.
e) Store at 4ºC until use. Use this buffer prepared before 1 hour.

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

1. Pipet **50 µl Proteinase K** into a sterile centrifuge microtube .

2. **Add 200 µl of plasma or serum** into microtube. If you process samples of <200 µl, adjust the final volume to 200 µl using PBS or 0.9% NaCl.

3. **Add 200 µl Viral Lysis Buffer (containing the carrier RNA).** Close the microtube and vortex vigorously for 20 seconds.

4. **Incubate at 70ºC for 10 minutes.**

5. Briefly centrifuge the microtube to collect the drops from the cap.

6. **Add 250 µl ethanol 100% to the sample.** Vortex for 15 seconds.

7. **Add the sample into the Spin Column** with a collection tuve.

8. **Centrifuge at 10.000 rpm for 1 min.** Remove the collection tube. Place the spin column in a new collection tube.

9. **Wash the column with 500 µl Wash Virus Buffer 1 with ethanol.** Centrifuge the column at 12,000 rpm for 1 min.

10. **Wash the column with 500 µl Wash Virus Buffer 2 with ethanol.** Centrifuge the column at 12,000 rpm for 1 min.

11. **Wash the column with 500 µl Wash Virus Buffer 2 with ethanol.** Centrifuge the column at 12,000 rpm for 1 min.

12. **Centrifuge the spin column at full speed for 2 minutes** to remove residual ethanol.

13. **Elute with 30-50 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 30-50 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