DANAGENE SALIVA RNA KIT

Ref.0809.1 50 preps

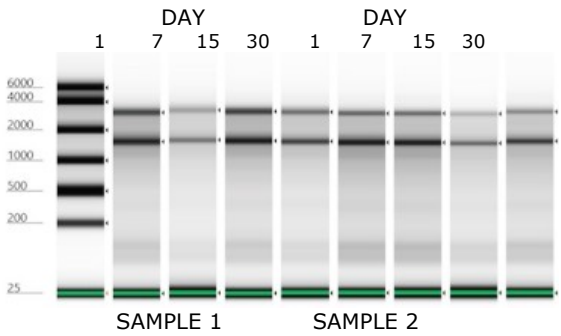
1. INTRODUCTION

DANAGENE SALIVA RNA Kit has been designed for a fast and efficient purification of total RNA from preserved saliva samples in our **DANASALIVA RNA Sample Collection Kit.**

The process includes a cell lysis with proteinase K followed by a precipitation of the proteins and part of genomic DNA. Later, by a precipitation with isopropanol, total RNA is obtained, which is finally rehydrated. Finally, for removal of genomic DNA contamination is used an approach consisting of two sequential filtrations with different MiroSpin columns.

Features:

- **Buffer-based RNA isolation combined with gDNA removal with columns..**
- **Sample Volume: 600 ul of preserved saliva sample.**
- **RNA is isolated without the use of harmful chemicals as phenol or chloroform..**
- **A260 / 280: > 1.8**
- **Elution Volume: 50 ul**



Purified total RNA is analyzed by the Agilent 4200 TapeStation System

2. KIT COMPONENTS

	50 preps	Stock
Precipitation Buffer	**12 ml**	Room temperature
Binding Buffer	**22 ml**	Room temperature
Wash Buffer*	**10 ml**	Room temperature
Nuclease-free water	**8 ml**	Room temperature
Proteinase K*	**30 mg**	-20ºC
gDNA Removal Columns	**50 units**	Room temperature
RNA columns	**50 units**	Room temperature
Collection Tubes	**100 units**	Room temperature

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

2.1Equipment and additional reagents required

- Microcentrifuge.
- Microtubes of 1. 5 ml and 2.0 ml.
- Ethanol 70%.
- Isopropanol.
- Water bath, dry bath or block with heating (90ºC).

3. PROTOCOL

3.1 Preliminary Preparations

- Dissolve the proteinase K in **1.3 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 40 ml of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.

3.2 Protocol for total RNA isolation from preseverd saliva samples

Tota RNA isolation

1. Mix well the tube containing 1 ml of preserved saliva sample in the DANASALIVA RNA Sample Collection Kit. **It is important** to see a homogeneous solution.

2. In a microtube with **25 ul of Proteinase K** add **600 ul of preserved saliva**. **Incubate at 55ºC for 45 minutes.**

3. Incubate at **90ºC for 15 minutes.**

4. Place the microtubes at 4ºC for 10 minutes.

5. Add **200 µl Precipitation Buffer. Vortex.**

6. **Centrifuge at 14.000 rpm for 5 minutes**.

7. Pour the **supernatant** in a new 1.5 ml microtube containing **600 µl of Isopropanol.** Avoid catching the possible superficial layer and / or pellet that can be formed.

8. **Centrifuge at 14.000 rpm for 3 minutes.** The RNA will be visible as a white pellet.

9. Remove the supernatant. Add **600 µl of Ethanol 70 %** and invert the tube several times to wash the RNA pellet.

10. **Centrifuge at 14.000 rpm for 1 minute.** Carefully remove the supernatant without touching the RNA pellet. It can be re-centrifuged briefly to collect the last drops of residual ethanol.

Removal gDNA contaminant

1. **Add 400 µl of Binding Buffer** . Resuspend well the RNA pellet with micropipette.

2. **Incubate at 55ºC for 15 minutes**.

3. **Transfer the sample to a gDNA removal column.** Place the column into a collection tube.

4. **Centrifuge for 1 minute at 10.000 r.p.m.**

5. Add **400 µl de ethanol 100% to the supernatant collected in point 4.** Mix well

6. Take one **RNA column and its collection tube** and add the mixture from point 5. Centrifuge at **8.000 -10.000 rpm** for **60 seconds.** Pass the sample twice as the volume exceeds the capacity of the column.

7. Add **700 µl Wash Buffer.** Centrifuge at maximum speed for 1 minute.

8. 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.

9. Add **50 µl of Nuclease-Free water.** Incubate for 2 minutes and centrifuge **at maximum speed for 1 minute.**

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es