Protocol for total RNA isolation and genomic DNA from preserved 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 DNA/RNA pellet. It can be re-centrifuged briefly to collect the last drops of residual ethanol.

Removal gDNA contaminant

1. Add **100 µl of Nuclease-free-water** to the DNA/RNA pellet and resuspend by micropipette.

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

3. **Add 400 µl of Binding Buffer** + **50 µl of ethanol 100%.** Mix well.

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

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

6. Add **400 µl de ethanol 100% to the supernatant collected in point 5.** Mix well. **Save the gDNA removal column.**

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

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

9. 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.

10. Add **30-50 µl of Nuclease-Free water.** Incubate for 2 minutes and centrifuge **at maximum speed for 1 minute.**

Genomic DNA isolation

1. Add **700 µl etanol 80%** to **the gDNA removal column-**

2. Centrifuge at maximum speed for 1 minute.

3. **Centrifugar 3 minutos a máxima velocidad** para eliminar todo el etanol.

4. 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.

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