DANAGENE SALIVA KIT

QUICK PROTOCOL

Extraction from samples of 600 µl of direct saliva

Kit components

	Ref.0603.4 50 extractions	Ref.0603.41 160 extractions
Lysis buffer	30 ml	3 x 34,5 ml
Protein precipitation buffer	10 ml	3 x 14 ml
Hydration buffer	35 ml	3 x 37,5 ml

Equipment and reagents required and not provided
- Isopropanol.
- Ethanol 70%.
- Microtubes of 1,5 or 2 ml.
- High speed centrifuge.
- Vortex type mixer.
- Micropipette and tips.
- Water bath.

Sampling
Place approximately **1.5 ml of saliva** in a collection container. It is recommended not to have eaten anything during the 30 minutes prior to sampling. To do this pass the tongue with movements up-down by the walls of the cheeks, jaws and palate to collect the cells.

Cell Lysis
1. Place **600-800 µl of saliva** in a 1.5 ml microtube. Centrifuge for 90 seconds at 13,000-16,000 x g. Remove the supernatant with pipette without damaging the visible white cell pellet.

2. Add **600 µl of Lysis Buffer**. Resuspend with the micropipette by means of movements up and down, to dissolve the pellet of cells and to lyse it.
VERY IMPORTANT, resuspend completely the pellet without any white clump in the solution, otherwise the amount of DNA obtained will be small.

3. Incubate for 10 minutes. Shake the tubes gently every 2 minutes. If it is possible to incubate at 37°C, this will improve performance.

Protein precipitation.
1. Add **200 µl Protein Precipitation Buffer** to the cell lysate.

2. Mix vigorously with a vortex type mixer, at maximum speed for 20-30 seconds.

3. Centrifuge at 13,000-16,000 x g for 5 minutes. A precipitate will form. If floating particles are observed, re-centrifuge after incubating 5 minutes on ice.

DNA precipitation.
1. Transfer the supernatant containing the DNA to a 1.5 ml microtube containing **600 µl Isopropanol**.

2. Mix by inversion about 25-50 times.

3. Centrifuge at 13,000-16,000 x g for 2 minutes. The DNA will be visible as a white pellet.

4. Remove the supernatant and dry the tube briefly on absorbent paper. Add **600 µl of Ethanol 70%** to wash the DNA.

5. Centrifuge at 13,000-16,000 x g for 1 minute. Carefully remove the supernatant without touching the DNA pellet. Turn again briefly and with a micropipette and fine tip collect the last drops residual ethanol.

6. Invert the microtube into an absorbent paper and let it dry for about 5-10 minutes.

Hydration of DNA.
1. Add **100-750 µl of Hydration Buffer**, depending on the size of the DNA pellet, and resuspend with micropipette. It is possible that large pellets require incubation at 55°C for 1 hour in order to be able to resuspend completely them before carrying out the PCR. For very small pellets you can use **25-50 µl of Hydration Buffer.**

2. Store at 2-8°C. For long storage, store at -20°C or -80°C.

For any further questions or queries, please contact us at info@bioted.es