DANAGENE SALIVA KIT

Ref.0603.4 50 Isolations / Ref.0603.41 160 Isolations

1.INTRODUCCION

DANAGENE SALIVA Kit designed for an efficient and fast purification of highly pure genomic DNA from a wide variety of **saliva samples** including:

1. Saliva samples.
2. Buccal cell samples via mouth swabs from **DANASALIVA** Hisopos. Ref.0602.42
3. Saliva samples via **DANASALIVA Sample Collection Kit.** Ref.0603.43
4. Saliva samples via ORAGENE self collection kits (DNAGenotek).

The process includes a cell lysis with an anionic detergent that solubilizes the necessary cell components, the contaminant RNA can be removed with a RNase treatment. The cell proteins are removed by precipitation, which allows to leave the genomic DNA in solution. Finally, the genomic DNA is isolated by a precipitation with isopropanol.

Features:

- **DNA from saliva is equivalent to DNA from blood for downstream applications.**
- **Improve patient care and compliance with painless, non-invasive sample collection and decreases costs.**
- **Reproducible, fast and non-expensive method.**
- **Safe method, as it removes completely the need of using toxic reagents.**

Applications: DNA purified using **DANAGENE SALIVA KIT** is highly stable and suited for use in a wide range of applications such as:

- **DNA archiving.**
- **PCR and quantitative real-time PCR.**
- **SNP analysis.**
- **Southern Blotting.**
- **Next Generation Sequencing.**

In case of saliva, the DNA obtained from one only person shows an intra-subject variability, this is, there is not an estimated DNA/ ml quantity as it can change depending on the moment of collecting the sample. It may happen that the obtained DNA quantity is not very big and it will not be possible to carry on with later applications which need a big amount of DNA.

2.COMPONENTS KIT

	Ref. 0603.4	Ref. 0603.41	
Lysis Solution	40 ml	105 ml	RT
Protein Precipitation Solution	35 ml	85 ml	RT
DNA Hydratation Solution	40 ml	50 ml	RT
Rnase	3.25 µg	8 µg	-20ºC
Proteinase K	6.5 µg	16 µg	-20ºC

SAMPLES	Ref. 0603.4	Ref. 0603.41
600 µl de saliva sample	50 isolations	160 isolations
Buccal swabs with DANASALIVA HISOPOS Ref.0617.1	50 isolations	160 isolations
DANASALIVA Sample Collection Kit For processing 600 µl saliva sample	50 isolations	160 isolations
DANASALIVA Sample Collection Kit For processing 2.0 ml de saliva	20 isolations	50 isolations
ORAGENE self collection kits (DNAGenotek) OG-500	20 isolations	50 isolations
ORAGENE self collection kits (DNAGenotek) OG-501	40 isolations	100 isolations

Equipment and additional reagents

- ✓ Isopropanol.
- ✓ 70%Ethanol.
- ✓ 1.5 ml and 2.0 ml microtubes, 15 or 50 ml centrifuge tubes.
- ✓ Microcentrifuge or clinic centrifuge.
- ✓ Vortex.
- ✓ Water bath.

3.PROTOCOL

3.1 Preparation working solutions

- If the Lysis Solution contains a precipitate due to the low temperatures, incubate at 37ºC and mix to dissolve the precipitate.

- **Dissolve the proteinase K** in **325 µl** (50 isolations) or in **810 µl** (160 isolations) of nuclease-free water and store at –20ºC. It is recommended to do several aliquots to avoid to many thaw/freeze cycles. At this temperature it is stable for 1 year.

- **Dissolve the RNase** in **325 µl** (50 isolations) or in **810 µl** (160 isolations) of nuclease-free water and store at –20ºC. It is recommended to do several aliquots to avoid to many thaw/freeze cycles. At this temperature it is stable for 1 year.

3.2 Protocol for DNA isolation from 600 µl saliva samples

Saliva samples are to be collected before meals, wash mouth with water and wait about 30 minutes to collect the sample. Saliva samples can be processed directly or stored at 4 ° C if they are processed in less than 2 hours.

Cell Lysis

1. Centrifuge **600 µl saliva sample** for 90 seconds. Remove the supernatant using a pipette and avoiding damaging the cell visible white pellet.

2. Add **600 µl Lysis Solution + 5 µl** de **Proteinase K + 5 µl de RNase** and resuspend with pipette to lyse the cells. It is very important to observe that the solution is homogeneous without groups of cells.

3. **Incubate at 37ºC for 30-60 minutes.** If it is possible vortex periodically .

Protein precipitation.

1. Cool sample to -20ºC for 3 minutes.

2. Add **250 µl of Protein precipitation solution** to the cell lysate.

3. Vortex vigorously at maximum speed for 20-30 seconds.

4. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A white precipitate will be formed. If there are particles floating in the supernatant centrifuge again once you have incubated in ice for 5 minutes.

DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing **600 µl of Isopropanol**.

2. Mix turning the tube upside down for about 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 on absorbent paper. Add **600 µl of Ethanol 70%** to wash the DNA.

5. Centrifuge at 13.000-16.000 xg for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5 minutes.

DNA Hydratation

1. **Add 250-750 µl of DNA Hydratation Solution** and mix well with pipette.

2. Incubate at 50ºC for 1 hour for to help DNA solubilisation, or incubate “overnight” a room temperature with gentle mixing.

3. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

3.3 Protocol for DNA isolation from buccal swabs with DANASALIVA HISOPOS (Ref.0617.1)

Sample collection

1. It is recommended that the individual who is going to take the sample abstain from drinking coffee and take some food at least 30 minutes prior to collection. If this is not possible we recommend gentle washing with water only in the mouth.

2. Collect the sample of buccal cells with the swab. Rub the swab on the inside of the cheek (buccal wall) and gums with firm pressure about 20 times on each side of the face and each side of the swab.

3. Use immediately for DNA isolation. If it has to transport the sample, to leave the brush to dry at room temperature for 30 minutes. Then insert the brush into the receptacle is provided for shipping. In this tube container the sample may remain for 1 week at 22-37 ° C prior to make the DNA isoaltion. For longer storage keep the sample in the container at -20 ° C up to 6 months.

4. The use of DANASALIVA HISOPOS are suitable as they allow to recover almost all of the lysis buffer after the incubation period.

Cell lysis

1. Add **600 µl of Lisis Solution** in a 1.5 ml microtube. Cutting the brush head and handle some filling into the microtube. Vortex vigorously to release the cells from the brush.

2. Add **5 µl** de **Proteinase K (20 mg/ml)** + **5 µl de RNase** . Mix.

3. **Incubate at 37ºC for 60 minutes.** If it is possible vortex periodically .

4. Remove the brush head of the lysis solution, rubbing against the walls to collect the maximum amount of liquid.

Protein precipitation.

1. Cool sample to -20ºC for 3 minutes.

2. Add **250 µl of Protein precipitation solution** to the cell lysate.

3. Vortex vigorously at maximum speed for 20-30 seconds.

4. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A white precipitate will be formed.

DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing **600 µl of Isopropanol**.

2. Mix turning the tube upside down for about 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 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, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5 minutes.

DNA Hydratation

1. **Add 30-50 µl of DNA Hydratation Solution** and mix well with pipette.

2. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

3.4 Isolation from preserved saliva samples with the DANASALIVA Sample Collection Kit (Ref. 0603.43)

2 OPTIONS: It will depend on the required amount of DNA for subsequent application or preference of working with a microcentrifuge with 1. 5 ml microtubes (option A) or a clinical centrifuge with 15 ml tubes (option B).

A) To process 1.2 ml (saliva + saliva preservation solution) this representing approximately to process 600 ul of saliva.

Cell Lysis

1. In the DANASALIVA Sample Collection Kit it will be appreciated a white pellet containing the buccal cells . Shake the tube containing 2 ml of the collected saliva. **It is important to observe a homogeneous solution before to take 1.2 ml of sample.**

2. Centrifuge **1.2 ml (saliva + saliva preservation solution)** for 90 seconds at 13.000-16.000 x g. Remove the supernatant using a pipette and avoiding damaging the cell visible white pellet.

3. Add **600 µl Lysis Solution + 5 µl** de **Proteinase K + 5 µl de RNase** and resuspend with pipette to lyse the cells. It is very important to observe that the solution is homogeneous without groups of cells.

4. **Incubate at 37ºC for 30-60 minutes.** If it is possible vortex periodically .

Protein precipitation.

1. Cool sample to -20ºC for 3 minutes.

2. Add **250 µl of Protein precipitation solution** to the cell lysate.

3. Vortex vigorously at maximum speed for 20-30 seconds.

4. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A white precipitate will be formed. If there are particles floating in the supernatant centrifuge again once you have incubated in ice for 5 minutes.

DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing **600 µl of Isopropanol**.

2. Mix turning the tube upside down for about 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 on absorbent paper. Add **600 µl of Ethanol 70%** to wash the DNA.

5. Centrifuge at 13.000-16.000 xg for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5-10 minutes.

DNA Hydratation

1. **Add 250-750 µl of DNA Hydratation Solution** and mix well with pipette.

2. Incubate at 50ºC for 1 hour for to help DNA solubilisation, or incubate “overnight” a room temperature with gentle mixing.

3. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

B)To process all content of DANASALIVA Sample Colletion Kit (saliva + saliva preservation solution)

Cell Lysis

1. In the DANASALIVA Sample Collection Kit it will be appreciated a white pellet containing the buccal cells . Shake the tube containing 2 ml of the collected saliva. **It is important to observe a homogeneous solution.**

2. Transfer all content **of the tube (4,50 ml)** DANASALIVA Sample Collection Kit into a **15 ml centrifuge tube**. Ensure that all content has been transferred including white pellet.

3. **Centrifuge at 4000 rpm for 2 minutes.** Remove the supernatant avoiding damaging the cell visible white pellet.

4. Add **2 ml of Lysis Solution + 15 µl** de **Proteinase K** + **15 µl de RNase** and resuspend with pipette to lyse the cells. It is very important to observe that the solution is homogeneous without groups of cells.

5. **Incubate at 37ºC for 30-60 minutes.** If it is possible vortex periodically .

Protein precipitation.

1. Cool sample to -20C for 3 minutes.

2. Add **750 µl of Protein precipitation solution** to the cell lysate.

3. Vortex vigorously at maximum speed for 20-30 seconds.

4. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A white precipitate will be formed. If there are particles floating in the supernatant centrifuge again once you have incubated in ice for 5 minutes.

DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing **2.0 ml of Isopropanol**.

2. Mix turning the tube upside down for about 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 on absorbent paper. Add **2.0 ml of Ethanol 70%** to wash the DNA.

5. Centrifuge at 13.000-16.000 xg for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5-10 minutes.

DNA Hydratation

1. **Add 750-1000 µl of DNA Hydratation Solution** and mix well with pipette.

2. Incubate at 50ºC for 1 hour for to help DNA solubilisation, or incubate “overnight” a room temperature with gentle mixing.

3. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

3.5 Isolation from preserved samples with the ORAGENE self collection kits (DNAGenotek). OG-500 (2ml saliva) / OG-501 (1ml saliva)

Cell Lysis
1. Incubate ORAGENE/saliva samples at 50ºC in a water incubate for a mínimum of 1 hour or in an air incubator for a minimum of 2 hours.

2. Transfer lysate sample **(4 ml / 2 ml) to 15 ml centrifuge tube**.

3. Add (**1 ml / 0,50 ml) of Lysis solution** + **(15 µl / 7,50 µl) de RNase** . Vortex at high speed for10 seconds to mix samples and incubate 15 minutes at room temperature.

Protein precipitation
1. Add (**1.60 ml / 0,80 ml) Protein precipitation solution** to the cell lysate.

2. Vortex vigorously at maximum speed for 20-30 seconds.

3. Centrifuge at 4.000 rpm for 5 minutes. A white precipitate will be formed. If there are particles floating in the supernatant centrifuge again once you have incubated in ice for 5 minutes.

DNA Precipitation.
1. Transfer the supernatant containing the DNA into a new **de 15 ml** tube containing (**5 ml / 2,50 ml) Isopropanol.**

2. Mix turning the tube upside down for about 25 times.

3. Centrifuge at 4.000 rpm for 3 minutes. The DNA will be visible as a white pellet.

4. Remove the supernatant and dry the tube on absorbent paper. Add (**5 ml / 2,50 ml) of Ethanol 70%** to wash the DNA.

5. Centrifuge at 4.000 rpm for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5-10 minutes.

DNA Hydratation
1. Add (**750-1.000 µl** / 350-500 **µl**) **of DNA Hydratation Solution** and mix well with pipette.

2. Incubate at 50ºC for 1 hour for to help DNA solubilisation, or incubate "overnight" a room temperature with gentle mixing.

3. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubt or additional consultation on the protocol, do not hesitate to contact with the technical service of DANAGEN-BIOTED S.L info@danagen.es