DANAGENE SPIN SALIVA DNA KIT

Ref.0603.SPIN50 50 PREPS
Ref.0603.SPIN250 250 PREPS

1. INTRODUCTION

DANAGENE SPIN SALIVA DNA kit is designed for the rapid purification of **highly pure genomic DNA from saliva samples** using silica-based system with a MicroSpin format:

a) Preserved saliva samples in the DANASALIVA Sample Collection Kit.

b) Fresh saliva samples.

2. KIT COMPONENTS

	50 PREPS	250 PREPS	Tª Stock
Lysis Buffer PS	**35 ml**	**160 ml**	Room temperature
Desinibition Buffer *	**18 ml**	**82.50 ml**	Room temperature
Wash Buffer *	**10 ml**	**50 ml**	Room temperature
Elution Bufer	**6 ml**	**30 ml**	Room temperature
Proteinase K*	**30 mg**	**5 x 30 mg**	-20ºC
MicroSpin columnas	**50 units**	**250 units**	Room temperature
Collection Tubes	**100 units**	**500 units**	Room temperature

(*) These solution must be prepared as indicated in the Preliminary Preparations section of the protocol.
PRECAUTIONS: The Desinibition Buffer contains guanidium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined bleach.
Intended Use
All DANAGENE products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

2.1 Equipment and additional reagents required

Reagents
• 96 – 100 % ethanol

Consumables
• 1.5 mL microcentrifuge tubes
• Disposable pipette tips

Equipment
• Manual pipettors
• Heat block, dry bath, or water bath (70°C)
• Centrifuge for microcentrifuge tubes
• Personal protection equipment (e.g., lab coat, gloves, goggles)

2.1 Storage and stability

All components are stable for 12 months from the date of purchase being stored correctly and at room temperature (15-25ºC).

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 10 ml (50 preps) or 50 ml (250 preps) of Ethanol 100 %** to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.
- **Add 40 ml (50 preps) or 200 ml (250 preps) of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.
- Pre-heat the Elution Buffer at 70ºC.

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

1. Vortex DANASALIVA Sample Collection Kit tube containing preserved saliva sample in order to homogenate the sample correctly. Transfer **400 µl in a 1.5 ml microtube.**
2. **Add 600 µl Lysis Buffer PS + 25µl Proteinase K.** Vortex.
3. Incubate **at 55ºC for 20 minutes** with vortex periodically.
4. Centrifuge **at 14.000 rpm** for **2 minutes.**
5. Transfer **supernatant** in a new 1.5 ml microtube avoiding touching the pellet.
6. **Add 300 µl of Ethanol 100%.** Mix well.
7. Load **650 µl** mixture sample into reservoir of a combined MicroSpin column–collection tube assembly. **Centrifuge at 8.000 rpm for 30 seconds**.

8. Discard the flow-through and place the Spin Column back into the same 2 ml Collection Tube, repeat step 6 with the remaining sample mixture.

9. Carefully place the Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the Spin Column.

10. Add **500 µl of Desinhibition Buffer. Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

11. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

12. **Dry silica membrane**. Centrifuge at 14.000 rpm for 3 minutes.

12.Place the MicroSpin Column into a 1.5 mL nuclease-free tube (not provided) and add **50µL Pre-heat the Elution Buffer** at 70ºC on the centre of the white filter membrane. Incubate **at room temperature** for **2 minutes**.

13. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The DNA is now ready for downstream applications.

3.3 Protocol for genomic DNA isolation from 600-800 µl of fresh saliva

NOTE: Do **NOT** eat, drink, smoke or chew gum for 30 minutes before giving your saliva sample.

Fresh saliva samples must be processed immediately or keep at 4ºC if they will be processed in less than 2 hours.

1.Centrifuge **600-800 µl of saliva at 14.000 rpm** for 90 seconds. Remove the supernatant with micropipette without damaging the visible white pellet of cells.

If the cell pellet is very small, you can add another **600 µl of saliva** and repeat point 1.

Genomic DNA yield is proportional to the size of the cell pellet, the larger the pellets, the greater the amount of DNA obtained.

2. **Add 600 µl Lysis Buffer PS + 25µl Proteinase K.** Resuspend the cell pellet well using a micropipette**. Incubate at 55ºC for 20 minutes** with vortex periodically.

3. Centrifuge **at 14.000 rpm** for **2 minutes.**

4. Transfer **supernatant** in a new 1.5 ml microtube avoiding touching the pellet.

5. Add 100 µl of Ethanol 100%. Mix well.

6. Load lysate mixture sample into reservoir of a combined MicroSpin column–collection tube assembly. **Centrifuge at 8.000 rpm for 30 seconds**.

7. Carefully place the Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the Spin Column.

8. Add **500 µl of Desinhibition Buffer. Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

9. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

10. **Dry silica membrane**. Centrifuge at 14.000 rpm for 3 minutes.

11. Place the MicroSpin Column into a 1.5 mL nuclease-free tube (not provided) and add **50-100 µl Pre-heat the Elution Buffer** at 70ºC on the centre of the white filter membrane. Incubate **at room temperature** for **2 minutes**.

12. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The DNA is now ready for downstream applications.

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