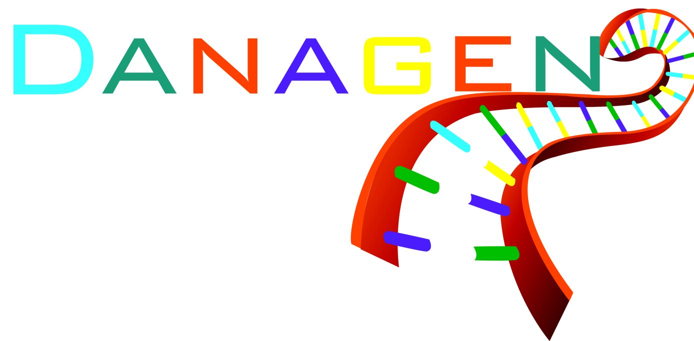

DANAGENE microSPIN DNA KIT

Ref. 0607.1 50 isolations
Ref. 0607.2 250 isolations

1.INTRODUCTION

This kit is designed for the efficient isolation of **genomic and mitochondrial DNA from small samples, such as different kinds of cells and tissues, laser-microdissected samples, small amounts of blood** using a special column design.

The special column design is connected with a reduced dead volume which **allows elution in as little as 10 µl.**

SAMPLE	SIZE
Tissues samples	< 10 mg
Cultured cells	< 10^5
Blood samples	< 100 µl
Laser-Microdissected tisssues	One sample
Buccal swab	one

MicroSpin Column Normal Column

Features:

- **Silica-membrane technology with specials MicroSpin columns.**
- **Rapid purification of high-quality DNA from small samples quantities.**
- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Elution volume: 10-30 µl.**
- **High quality DNA obtained that can be directly used in PCR, Southern, any enzymatic reaction, cloning, etc.**

Applications:

- **DNA isolation from tissue (e.g., mouse or human tissues, laser micro-dissections).**
- **DNA isolation from cells (e.g., cultured cells).**
- **DNA isolation from clinical samples (e.g., blood samples, biopsy samples).**
- **DNA isolation from forensic samples (e.g., dried blood spots, buccal swabs).**

2. KIT COMPONENTS

	Ref.0607.1	Ref.0607.2	Storage
RBC Lysis Solution	8 ml	40 ml	Room Temperature
Tissues Lysis Buffer	10 ml	50 ml	Room Temperature
Lysis Binding Buffer	10 ml	50 ml	Room Temperature
Proteinase K (*)	22 mg	105 mg	-20ºC
Desinhibition Buffer (*)	18 ml	85 ml	Room Temperature
Wash Buffer (*)	6 ml	30 ml	Room Temperature
Elution Buffer	2 ml	10 ml	Room Temperature
MicroSpin Columns	50	250	Room Temperature
Recollection Tube 2.0 ml	100 units	500 units	Room Temperature

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

3. PROTOCOL

3.1 Preliminary Preparations

- Both the Lysis/ Binding Buffer and the Desinhibition Buffer contain Guanidine hydrochloride which is an irritant agent , for this reason we recommend to use gloves and glasses for its manipulation.
- Dissolve the proteinase K in **1.1 ml** (50 extractions) or in **5.2 ml** (250 extractions) 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.
- Verify that the Tissues Lysis Buffer or the Lysis/ Binding Buffer do not have precipitates due to the low temperatures. If necessary, dissolve heating at 37ºC.
- Add **10 ml** (50 extractions) or **50 ml** (250 extractions) of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation
- Add **24 ml** (50 extractions) or **120 ml** (250 extractions) 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 Protocol for genomic DNA extraction from Blood

1. Add **30-50 µl** of whole blood to a microtube containing 150 µl of **RBC Lysis Solution** . Mix and incubate for 10 minutes at room Tª. Turn the tube upside down several times during the incubation period.

2. Centrifuge for 20-30 seconds at 13.000-16.000 x g. Remove the supernatant using a pipette and avoiding damaging the cell visible pellet and leaving 10-20 µl of residual liquid. Vortex the microtube to resuspend the pellet.

3. Add **180 µl of the Tissue Lysis Buffer** + **20 µl Proteinase K.** Mix well by vortex.

4. **Incubate at 56ºC** for 1-4 hour or until the lysis is complete, the samples can be incubated overnight. The samples which are difficult to lyse can be ground under liquid Nitrogen or can be directly treated in a mechanical homogenizer (Polytrom, Ultra Turrax).

5. Add **200 µl of Lysis/Binding Buffer.** Mix by vortexing. **Incubate at 70ºC for 10 minutes**. If there are insoluble particles, centrifuge 5 minutes at maximum speed and pour the supernatant into a new microtube.

6. Add **200 µl of Ethanol (96-100%) to the lysate.** Mix by vortexing. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

7. **Transfer the lysate** into reservoir of a combined MicroSpin Column –collection tube assembly without wetting the rim.

8. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not drawn completely through the matrix, repeat the centrifugation step.

9. Place the MicroSpin column in a new collection tube and add **500 µl of Deshinibition Buffer** to the reservoir without wetting the rim.

10. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

11. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column without wetting the rim. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

12. Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.

13. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**.** **Add 10- 30 µl of Elution Buffer** (preheated at 70ºC) directly onto the center of the silica membrane. **Incubate 1 minute.**

14. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

3.3 Protocol for genomic DNA extraction from cultured cells

1. Resuspended up to **10^5 cells** in a final volume of **80 µl of the Tissue Lysis Buffer** + **10 µl Proteinase K.** Mix by vortexing 2-5 seconds.

2. **Incubate at 56ºC for 10 min.**

3. Add **80 µl of Lysis/Binding Buffer.** Mix by vortexing. **Incubate at 70ºC for 5 minutes**.
   Let the lysate cool down to room temperature.

4. Add **80 µl of Ethanol (96-100%) to the lysate.** Mix by vortexing. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

5. **Transfer the lysate** into reservoir of a combined MicroSpin Column –collection tube assembly without wetting the rim.

6. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not Drawn completely through the matrix, repeat the centrifugation step.

7. Place the MicroSpin column in a new collection tube and add **80 µl of Deshinibition Buffer** to the reservoir without wetting the rim.

8. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

9. **Add 80 µl of Wash Buffer** into reservoir of MicroSpin column without wetting the rim. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

10. Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.

11. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**.** **Add 10- 30 µl of Elution Buffer** (preheated at 70ºC) directly onto the center of the silica membrane. **Incubate 1 minute.**

12. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA

3.4 Protocol for genomic DNA extraction from less than 10 mg of Animal tissues

1. Transfer a tissue sample of less than 10 mg to a 1.5 ml microcentrifuge tube.

2. Add **180 µl of the Tissue Lysis Buffer** + **20 µl Proteinase K.** Mix well by vortex.

3. **Incubate at 56ºC** for 1-4 hour or until the lysis is complete, the samples can be incubated overnight. The samples which are difficult to lyse can be ground under liquid Nitrogen or can be directly treated in a mechanical homogenizer (Polytrom, Ultra Turrax).

4. Add **200 µl of Lysis/Binding Buffer.** Mix by vortexing. **Incubate at 70ºC for 10 minutes**. If there are insoluble particles, centrifuge 5 minutes at maximum speed and pour the supernatant into a new microtube.

5. Add **200 µl of Ethanol (96-100%) to the lysate.** Mix by vortexing. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

6. **Transfer the lysate** into reservoir of a combined MicroSpin Column –collection tube assembly without wetting the rim.

7. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not drawn completely through the matrix, repeat the centrifugation step.

8. Place the MicroSpin column in a new collection tube and add **500 µl of Deshinibition Buffer** to the reservoir without wetting the rim.

9. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

10. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column without wetting the rim. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

11. **Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.**

12. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**.**
    Add 10- 30 µl of Elution Buffer (preheated at 70ºC) directly onto the center of the silica membrane. **Incubate 1 minute.**

13. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

3.5 Protocol for genomic DNA extraction from laser-microdissected tissues

NOTE: This protocol the extraction of genomic DNA from laser-microdissected samples is a challenge for molecular analysis, the sample amount is very small and the DNA quality is adversely affected by fixation and staining procedures, and it may be necessary either to modify fixation protocols or to use cryosections from flash-frozen specimens to minimize this problem.

1. Place laser-microdiseccected sample into a 1.5 ml microcentrifuge tube.

2. Add **80 µl of the Tissue Lysis Buffer** + **10 µl Proteinase K.** Mix by vortexing 2-5 seconds.

3. **Incubate at 56ºC** for 1-4 hour or until the lysis is complete, the samples can be incubated overnight.
   The optimal incubation time may vary depending on sample type and amount.

4. Add **80 µl of Lysis/Binding Buffer.** Mix by vortexing. **Incubate at 70ºC for 5 minutes**.
   Let the lysate cool down to room temperature.

5. Add **80 µl of Ethanol (96-100%) to the lysate.** Mix by vortexing. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

6. **Transfer the lysate** into reservoir of a combined MicroSpin Column –collection tube assembly without wetting the rim.

7. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not Drawn completely through the matrix, repeat the centrifugation step.

8. Place the MicroSpin column in a new collection tube and add **80 µl of Deshinibition Buffer** To the reservoir without wetting the rim.

9. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

10. **Add 80 µl of Wash Buffer** into reservoir of MicroSpin column without wetting the rim. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

11. **Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.**

12. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**.** **Add 10- 30 µl of Elution Buffer** (preheated at 70ºC) directly onto the center of the silica membrane. **Incubate 1 minute.**

13. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

3.6 Protocol for DNA isolation from buccal swabs

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.

5. Add **250 µl of the Tissue Lysis Buffer + 20 µl Proteinase K** 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. Ensure that the entire sample is covered with the Buffer during incubation.

6. **Incubate at 56ºC for 30-60 minute.** Centrifuge briefly. Remove the brush head of the lysis solution, rubbing against the walls to collect the maximum amount of liquid. **Measuring the volume of lysate.**

5. Add an **equal volume of Lysis/Binding Buffer** to the lysate volume. Mix by vortexing. **Incubate at 70ºC for 10 minutes**.

6. Add an **equal volume of 100% Etanol** to the lysate volume. Mix by vortexing

7. **Transfer the lysate** into reservoir of a combined MicroSpin Column –collection tube assembly without wetting the rim.

8. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not drawn completely through the matrix, repeat the centrifugation step.

9. Place the MicroSpin column in a new collection tube and add **200 µl of Deshinibition Buffer** to the reservoir without wetting the rim.

10. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

11. **Add 200 µl of Wash Buffer** into reservoir of MicroSpin column without wetting the rim. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

12. **Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.**

13. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**.** **Add 10- 30 µl of Elution Buffer** (preheated at 70ºC) directly onto the center of the silica membrane. **Incubate 1 minute.**

14. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

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