DANAGENE SPIN GENOMIC DNA KIT

Ref.0605.1 50 extractions
Ref.0605.2 250 extractions
Ref.0605.3 1000 extractions

1. INTRODUCTION

This kit is designed for rapid extraction and purification of high quality genomic DNA from a wide variety of samples including whole blood, cultured cells, animal tissues, mouse tails, bacteria, yeasts, clinical samples (serum, plasma, stool, urine), forensic samples, and paraffin embedded tissues.

The kit contains enough reagents and columns to perform **50/250/1000 extractions of genomic DNA,** according to the kit, of different samples:

- 200 µl whole blood.
- 200 µl buffy coat.
- 10^4-10^6 cells in culture.
- 25-50 mg tissue.
- 0.2-0.5 cm mouse tail.
- 10^8 bacteria.
- 10^9 yeasts.
- Sections of tissues embedded in paraffin.

NOTE: For any other type of sample request the protocol to the technical service of DANAGEN-BIOTED at info@danagen.es.

The procedure includes lysis in **SDS** and **proteinase K**, then a chaotropic solution is added which will create the conditions necessary for the DNA to bind to the glass fiber membrane and finally the DNA is eluted with an **elution buffer**.

The DNA obtained is of high quality and can be used directly in PCR, Southern, cloning, any enzymatic reaction, etc.

2. KIT INCLUDES

Enough reagents to	50 extractions	250 extractions	1000 extractions	Store temperature
Tissues lysis buffer	10 ml	50 ml	4 x 50 ml	15-25ºC
Lysis/binding buffer	10 ml	50 ml	4 x 50 ml	15-25ºC
Proteinase K*	22 mg	105 mg	4 x 105 mg	-20ºC
Desinhibition buffer*	16,5 ml	82,5 ml	4 x 82,5 ml	15-25ºC
Wash buffer*	10 ml	50 ml	4 x 50 ml	15-25ºC
Elution buffer	10 ml	50 ml	4 x 50 ml	15-25ºC
MicroSpin Columns	50 units	250 units	1000 units	15-25ºC
Collection tubes	100 units	500 units	2000 units	15-25ºC

*** See in the section on Prelab preparations how to prepare these solutions**

Equipment and reagents required and not provided

- Isopropanol.
- Ethanol 100%.
- 1.5 ml microtubes.
- Microcentrifuge.
- Vortex type mixer.
- Water bath.

Storage and stability

All components are stable for 12 months from date of purchase being stored and used as indicated.

ATTENTION: The Lysis/binding buffer and Desinhibition buffer contain guanidine hydrochloride which is irritating, wear gloves and goggles.

3. PROTOCOL

The protocol involves the following steps:

- Samples are lysed with the appropriate **Lysis buffer** and **Proteinase K**.
- Nucleic acids bind to the fiberglass matrix packaged in the MicroSpin Columns.
- The nucleic acids are washed first with the **Desinhibition buffer** to remove the PCR inhibitors.
- Washing of nucleic acids to remove salts, proteins and other impurities.
- Nucleic acids are eluted.

3.1 PreLab Preparations

- Dissolve **Proteinase K** in **1.1 ml** of nuclease-free water (50 extractions kit) and **5.2 ml** (250 extractions kit) and store at -20°C. Several aliquots are recommended to avoid too many thaw-frozen cycles. At this temperature it is stable for 1 year.
- Check that the **Tissue lysis buffer**, **Lysis/binding buffer** have no precipitates due to the low temperatures. If necessary, dissolve by heating to 37°C.
- Add **Ethanol** 100% to the **Desinhibition buffer** indicated on the label, about **10 ml** (50 extractions kit) and about **50 ml** (250 extractions kit). Keep container tightly closed to prevent evaporation of **ethanol**.
- Add **Ethanol** 100% to the **Wash buffer** indicated on the label, about **40 ml** (50 extractions kit) and about **200 ml** (250 extractions kit). Keep container tightly closed to prevent evaporation of **ethanol**.

- **Preheating the Elution buffer to 70°C can increase the yield of DNA obtained. For some subsequent application it may be necessary for the DNA to be concentrated, elution in smaller volumes of 200 µl will increase the final concentration of DNA in the eluate but will reduce the overall yield of DNA obtained. For samples containing <3 µg DNA, an elution in 100 µl is recommended. For samples containing <1 µg DNA, an elution in 50 µl is recommended.**

3.2 Protocol for extraction of genomic DNA from whole blood, buffy coat and cells in culture.

- 200 µl total blood.
- 200 µl buffy coat.
- 10^4- 10^6 cells in culture.

If the material does not reach 200 µl, bring the final volume of the sample to 200 µl with nuclease-free water.

1. To 200 µl of the indicated material add 200 µl of the **Lysis/binding buffer + 20 µl Proteinase K**. Incubate at 70°C for 10 minutes.

2. Add **100 µl of Isopropanol**. Mix well.

3. Pipette the lysate into the reservoir of the Spin microcolumn with its collection tube. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube.

4. Place the MicroSpin column in a new collection tube and add 500 µl of **Desinhibition buffer** to the reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

5. Add **500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

6. 2nd Washing. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

7. **Centrifuge at maximum speed for 90 seconds to remove residual ethanol**.

8. Remove the collection tube and insert the microcolumn spin into a 1.5 ml microtube. **Add between 50-200 µl of Elution buffer** (preheated to 70 ° C) in the microcolumn spin reservoir. Incubate for 2 minutes.

9. **Centrifuge at full speed for 60 seconds**. The microtube now contains the genomic DNA.

3.3 Protocol of extraction of genomic DNA from 25 mg of animal tissues

1. Cut 25 mg of human or animal tissue into small pieces and place in a 1.5 ml microtube. Add **180 µl of the Tissue lysis buffer + 20 µl Proteinase K**. Mix thoroughly. Incubate at 55°C for 1 hour or until lysis is complete, samples may be incubated overnight.
Samples that are difficult to lyse can be milled with liquid Ni or can be treated directly with a Polytron type homogenizer.

2. Add **200 µl Lysis/binding buffer**. Shake with Vortex type mixer. Incubate at 70°C for 10 minutes. If insoluble particles are found, centrifuge 5 minutes at maximum speed and transfer the supernatant to a new microtube.

3. Add **100 µl of Isopropanol**. Mix well.

4. Pipette the lysate into the reservoir of the Spin microcolumn with its collection tube. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube.

5. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition buffer** to the reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

6. Add **500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

7. 2nd Washing. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

8. **Centrifuge at maximum speed for 90 seconds to remove residual ethanol**.

9. Remove the collection tube and insert the microcolumn spin into a 1.5 ml microtube. **Add between 50-200 µl of Elution buffer** (preheated to 70°C) in the microcolumn spin reservoir. Incubate for 2 minutes.

10. **Centrifuge at full speed for 60 seconds**. The microtube now contains the genomic DNA.

3.4 Protocol for extraction of genomic DNA from 25-50 mg of mouse tail

1. Cut 0.2-0.5 cm of mouse glue into several pieces and place in a 1.5 ml microtube. Add **180 µl of the Tissue lysis buffer + 20 µl Proteinase K**. Mix thoroughly. Incubate at 55°C until lysis is complete, samples can be incubated overnight. To remove residues of bones or hairs, centrifuge for 5 minutes at maximum speed and transfer the supernatant to a new microtube.

2. Add **200 µl of Lysis/binding buffer and 100 µl of Isopropanol**. Mix thoroughly with a vortex type mixer.

3. Pipette the lysate into the reservoir of the Spin microcolumn with its collection tube. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube.

4. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition buffer to the reservoir. Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

5. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

6. 2nd Washing. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

7. **Centrifuge at maximum speed for 90 seconds to remove residual ethanol**.

8. Remove the collection tube and insert the microcolumn spin into a 1.5 ml microtube. **Add between 50-200 µl of elution buffer** (preheated to 70°C) in the microcolumn spin reservoir. Incubate for 2 minutes.

9. **Centrifuge at full speed for 60 seconds**. The microtube now contains the genomic DNA.

3.5 Protocol for extracting of genomic DNA from 10^9 bacteria

1. Centrifuge 1-1.5 ml of bacterial culture. Remove the supernatant. Resuspend the pellet in **180 µl Tissue lysis buffer and then add 20 µl Proteinase K**. Shake with Vortex and incubate at 55°C until lysis is complete.

For those strains difficult to lyse, especially the Gram +, a previous incubation with lithic enzymes is necessary. Resuspend the pellet with 200 µl PBS with 20 mg/ml lysozyme and/or lysostaphin (10 mg/ml), incubate 30 minutes at 37°C, then add **20 µl Proteinase K** and incubate at 55°C until lysis is complete.

2. Add **200 µl Lysis/binding buffer**. Shake with Vortex type mixer. Incubate at 70°C for 10 minutes.

3. Add **100 µl of Isopropanol**. Mix well.

4. Pipette the lysate into the reservoir of the Spin microcolumn with its collection tube. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube.

5. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition buffer to the reservoir**. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

6. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

7. 2nd Washing. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds.** Remove the liquid.

8. **Centrifuge at maximum speed for 90 seconds to remove residual ethanol**.

9. Remove the collection tube and insert the microcolumn spin into a 1.5 ml microtube. **Add between 50-200 µl of Elution buffer** (preheated to 70°C) in the microcolumn spin reservoir. Incubate for 2 minutes.

10. **Centrifuge at full speed for 60 seconds**. The microtube now contains the genomic DNA.

3.6 Protocol for extraction of genomic DNA from 10^8 yeasts

1. Centrifuge 3 ml of yeast culture. Remove the supernatant. Resuspend the pellet in 290 µl of 50 mM EDTA and 10 µl of Liticasa (20 mg/ml). Incubate at 37°C for 30-60 minutes. Centrifuge at maximum speed and remove the supernatant. Resuspend the pellet with **180 µl Tissue lysis buffer and then add 20 µl Proteinase K**. Shake with Vortex type mixer. Incubate at 55ºC until lysis is complete.

2. Add **200 µl Lysis/binding buffer**. Shake with Vortex type mixer. Incubate at 70°C for 10 minutes.

3. Add **100 µl of Isopropanol**. Mix well.

4. Pipette the lysate into the reservoir of the Spin microcolumn with its collection tube. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube.

5. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition buffer to the reservoir. Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

6. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

7. 2nd Washing. **Add 500 µl of Wash buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

8. **Centrifuge at maximum speed for 90 seconds to remove residual ethanol**.

9. Remove the collection tube and insert the microcolumn spin into a 1.5 ml microtube. **Add between 50-200 µl of Elution buffer** (preheated to 70°C) in the microcolumn spin reservoir. Incubate for 2 minutes.

10. **Centrifuge at full speed for 60 seconds**. The microtube now contains the genomic DNA.

4. GUIDE TO PROBLEMS AND SOLUTIONS

For any doubt or additional consultation about the protocol, please contact DanaGen-BioTed Technical Service at info@danagen.es