



## DANAGENE SPIN GENOMIC DNA KIT

**Ref.0605.1 50 isolations**

**Ref.0605.2 250 isolations**

**Ref.0605.3 1000 isolations**

### 1. INTRODUCTION

This kit is designed for the rapid purification of highly pure genomic DNA from a wide variety of samples, including whole blood, culture cells, animal tissues, mouse tails, bacteria, yeasts, clinic samples (serum, plasma, feces, urine), forensic samples and paraffin-embedded tissues using **MicroSpin columns with glass fiber which selectively binds the DNA**.

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

- 200 µl whole blood.
- 200 µl buffy coat.
- 10<sup>4</sup>-10<sup>6</sup> culture cells.
- 25-50 mg tissue.
- 0.2-0.5 cm mouse tail.
- 10<sup>8</sup> bacteria.
- 10<sup>9</sup> yeast.
- Paraffin-embedded tissues.
- For any other kind of sample contact **DANAGEN-BIOTED** laboratory technical service and ask for the protocol.

The process includes a cell lysis by incubation of the sample in a solution containing SDS and proteinase K at 55°C. Appropriate conditions for binding of DNA to the glass fiber membrane are created by addition of large amounts of chaotropic ions to the lysate. Contaminants are removed by efficient washing with wash buffer. Pure genomic DNA is finally eluted with an elution buffer and it's ready to use for subsequent reactions.

### 2. KIT COMPONENTS

	50 isolations	250 isolations	1000 isolations	T <sup>3</sup> Stock
Tissue Lysis Buffer	10 ml	50 ml	4 x 50 ml	15-25°C
Lysis/ Binding Buffer	10 ml	50 ml	4 x 50 ml	15-25°C
Proteinase K <sup>(*)</sup>	22mg	105mg	4 x 105mg	-20°C
Desinhibition Buffer <sup>(*)</sup>	16.5 ml	82.5 ml	4 x 82.5 ml	15-25°C
Wash Buffer <sup>(*)</sup>	10 ml	50 ml	4 x 50 ml	15-25°C
Elution Buffer	10 ml	50 ml	4 x 50 ml	15-25°C
MicroSpin Columns	50 unid.	250 unid.	1000 unid.	15-25°C
Collection tubes	100 unid.	500 unid.	2000 unid.	15-25°C

<sup>(\*)</sup> These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

## 2.1 Equipment and additional reagents required

- Isopropanol.
- Ethanol 100 %
- Microcentrifuge.
- 1.5 ml microtubes

## 3. **PROTOCOL**

The protocol implies the following steps:

- The samples are lysed by incubation with special Lysis Buffer and proteinase K
- The nucleic acids are bound to the glass fibers matrix pre- packed in the MicroSpin Columns.
- The nucleic acids are first washed with the Desinhibition Buffer to remove the PCR inhibitors.
- The nucleic acids are washed to remove salts, proteins and other impurities.
- The nucleic acids are recovered using the Elution buffer.

### 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  $-20^{\circ}\text{C}$ . 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  $37^{\circ}\text{C}$ .
- Add **10 ml** (50 extractions) or **50 ml** (250 extractions) of Ethanol 100 % to the Desinhibition Buffer (REA03). Keep the container closed to avoid the ethanol evaporation
- Add **40 ml** (50 extractions) or **200 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  $70^{\circ}\text{C}$ . For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200  $\mu\text{l}$  increases the final DNA concentration in the eluate significantly but slightly reduces overall DNA yield . For samples containing  $<3 \mu\text{g}$  of DNA, eluting the DNA in 100  $\mu\text{l}$  is recommended. For samples containing less than 1  $\mu\text{g}$  of DNA, only one elution in 50  $\mu\text{l}$  is recommended.**

### 3.2 Protocol for genomic DNA extraction from whole blood, buffy coat and culture cells

- 200  $\mu\text{l}$  whole boold
- 200  $\mu\text{l}$  buffy coat.
- $10^4$ -  $10^6$  culture cells.

If your sample volume is less than 200  $\mu\text{l}$  , add nuclease-free water to the final volume of the sample until you reach 200  $\mu\text{l}$  .

1. Add **200  $\mu\text{l}$  of the Lysis/ Binding Buffer + 20  $\mu\text{l}$  Proteinase K** to 200  $\mu\text{l}$  of the sample material. Mix well. Incubate at  $70^{\circ}\text{C}$  for 10 minutes.
2. Add **100  $\mu\text{l}$  of Isopropanol**. Mix well.
3. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly.
4. **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  $\mu\text{l}$  of Desinhibition Buffer** to the reservoir. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
6. **Add 500  $\mu\text{l}$  of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
7. 2<sup>o</sup> Wash. **Add 500  $\mu\text{l}$  of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 14.000 rpm for 60 seconds**. Remove the liquid.
8. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol**.
9. Remove the collection tube and insert the MicroSpin Column in a 1.5 ml microtube . **Add 200  $\mu\text{l}$  of elution buffer** (preheated at  $70^{\circ}\text{C}$ ) into reservoir of MicroSpin column. Incubate 2 minutes.
10. **Centrifuge at maximum speed for 60 seconds**. The microtube contains now the genomic DNA.

### **3.3 Protocol for genomic DNA extraction from 25 mg of Animal tissues**

1. Cut 25 mg of animal or human tissue in small pieces and place them in 1.5 ml microtube. Add **180 µl of the Tissue Lysis Buffer + 20 µl Proteinase K**. Mix well. Incubate at 55°C for 1 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).
2. Add **200 µl of Lysis/Binding Buffer**. Shake with vortex. Incubate at 70°C for 10 minutes. If there are insoluble particles, centrifuge 5 minutes at maximum speed and pour the supernatant into a new microtube.
3. Add **100 µl of Isopropanol**. Mix well.
4. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly.
5. **Centrifuge at 8.000 rpm for 60 seconds**. Remove the collection tube.
6. 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.
7. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
8. 2° Wash. Add 500 µl of Wash Buffer into reservoir of MicroSpin column. Centrifuge at 14.000 rpm for 60 seconds. Remove the liquid.
9. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol**.
10. Remove the collection tube and insert the MicroSpin column in a 1.5 ml microtube. **Add 50-200 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 2 minutes.
11. **Centrifuge at maximum speed for 60 seconds**. The microtube contains now genomic DNA.

### **3.4 Protocol for Genomic DNA extraction from 25 -50 mg of mouse tail**

1. Cut 0.2-0.5 cm of mouse tail in several pieces and put them in a 1.5 ml microtube. Add **180 µl of the Tissue Lysis Buffer + 20 µl Proteinase K**. Mix well. Incubate at 55°C until the lysis is completed, the samples can be incubated overnight. To remove bone or hair remains, centrifuge for 5 minutes at maximum speed and pour the supernatant into a new microtube.
2. Add **200 µl of Lysis/Binding Buffer and 100 µl of Isopropanol**. Mix well with vortex.
3. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly.
4. **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** into reservoir of MicroSpin column. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
7. 2° Wash. Add 500 µl of Wash Buffer into reservoir of MicroSpin column. Centrifuge at 14.000 rpm for 60 seconds. Remove the liquid.
8. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol**.
9. Remove the collection tube and place the MicroSpin column in a 1.5ml. microtube. **Add 50-200 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 2 minutes.
10. **Centrifuge at maximum speed for 60 seconds**. The microtube contains now the genomic DNA.

### **3.5 Protocol for genomic DNA extraction from 10<sup>9</sup> bacteria**

1. Centrifuge 1-1.5 ml of bacteria culture. Remove the supernatant. Resuspend the pellet in **180 µl of Tissue Lysis Buffer and then add 20 µl Proteinase K**. Shake with vortex and incubate at 55°C until the lysis is completed.

For those strains which are difficult to lyse, specially Gram+ bacteria, it is necessary a previous incubation with lytic enzymes. Resuspend the pellet with 200 µl of PBS with 20 mg/ml of 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 the lysis is completed.

2. Add **200 µl of Lysis/Binding Buffer**. Shake with vortex. Incubate at 70°C for 10 minutes.
3. Add **100 µl of Isopropanol**. Mix well.
4. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly.
5. **Centrifuge at 8.000 rpm for 60 seconds**. Remove the collection tube.
6. Place the MicroSpin column in a new collection tube and **500 µl of Desinhibition Buffer** to the reservoir. **Centrifuge at 12.000 rpm for 60 seconds**. Remove all the liquid.
7. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
8. 2° Wash. Add 500 µl of Wash Buffer into reservoir of MicroSpin column. Centrifuge at 14.000 rpm for 60 seconds. Remove the liquid.
9. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol**.
10. Remove the collection tube and place the MicroSpin column in a 1.5ml. microtube. **Add 50-200 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 2 minutes.
11. **Centrifuge at maximum speed for 60 seconds**. The microtube contains now the genomic DNA.

### **3.6 Protocol for the extraction of genomic DNA from 10<sup>8</sup> yeasts**

1. Centrifuge 3 ml of yeast culture. Remove the supernatant. Resuspend the pellet in 290 µl of EDTA 50 mM and 10 µl of Lyticase (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 de Tissue Lysis Buffer and then add 20 µl Proteinase K**. Shake with vortex. Incubate at 55°C until the lysis is completed.
2. Add **200 µl of Lysis/ Binding Buffer**. Shake with vortex. Incubate at 70°C for 10 minutes.
3. Add **100 µl de Isopropanol**. Mix well.
4. Pipette the lysate into reservoir of a combined MicroSpin Column –collection tube assembly. **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**. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
6. **Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 12.000 rpm for 60 seconds**. Remove the liquid.
7. 2° Wash. Add 500 µl of Wash Buffer into reservoir of MicroSpin column. Centrifuge at 14.000 rpm for 60 seconds. Remove the liquid.
8. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol**.
9. Remove the collection tube and place the MicroSpin column in a 1.5ml. microtube. **Add 50-200 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 2 minutes.
10. **Centrifuge at maximum speed for 60 seconds**. The microtube contains now the 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](mailto:info@danagen.es)