DANAGENE SPIN BLOOD DNA KIT

Ref. 0606.1 50 Preps
Ref. 0606.2 250 Preps

1. INTRODUCTION

This kit is designed for the rapid purification **of highly pure genomic DNA from whole blood, serum, plasma and biologics fluids** using Spin columns with silica membrane, which selectively binds DNA.

This kit uses a new formulated lysis/binding buffer specific for DNA isolation of blood samples for high yields.

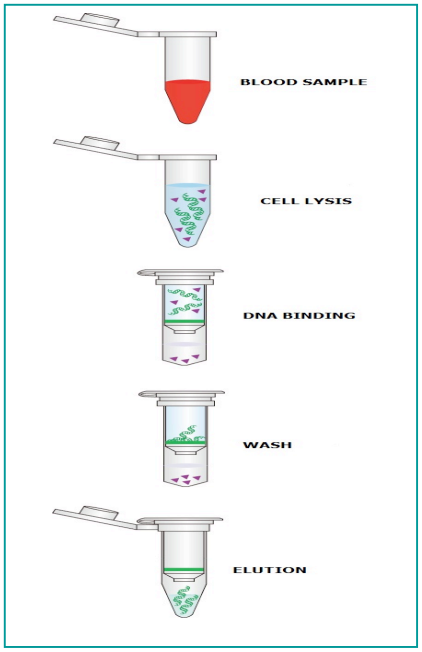
Features:

- **For rapid purification of high-quality, ready-to-use DNA from blood.**
- **Sample size: 300 µl whole blood, serum, plasma and body fluids.**
- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Typical yield: 6- 9 µg genomic DNA.**
- **Elution volume: 50-200 µl.**
- **High quality DNA obtained that can be directly used in PCR, Southern, any enzymatic reaction, cloning, etc.**

Applications:

- **Genomic, bacterial , viral DNA isolation.**
- **DNA from whole blood (human or animal blood, fresh or frozen).**
- **DNA from whole blood treated with citrate, EDTA, heparin.**
- **DNA from serum, plasma, buffy coat, platelets, body fluids, dried blood spots.**

Procedure: Lysis is achieved by incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of proteinase K at 70ºC. Appropriate conditions for binding DNA to the silica membrane are created by addition of ethanol to the lysate. Contaminants are removed by washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.



2. KIT COMPONENTS

Product	Ref.0606.1 50 Preps	Ref.0606.2 250 Preps	Store
RBC Lysis Buffer	50 ml	250 ml	15-25ºC
Lysis Tissue Buffer	10 ml	50 ml	15-25ºC
Lysis / Binding Buffer	15 ml	75 ml	15-25ºC
Proteinase K*	30 mg	2 x 75 mg	-20ºC
Desinhibition Buffer (*)	18 ml	90 ml	15-25ºC
Wash Buffer (*)	10 ml	50 ml	15-25ºC
Elution Buffer	10 ml	50 ml	15-25ºC
Spin Columns	50 units	250 units	15-25ºC
Collection tubes	100 units	500 units	15-25ºC

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

Equipment and reagents necessary and not provided

- 100% Ethanol.
- Microcentrifuge.
- Microtubes of 1.5 ml.

Storage and stability

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

3. PROTOCOL

3.1 Collection and storage of blood samples.

Whole blood samples should be refrigerated to 4ºC immediately after collection. They can also be shipped in refrigerated packaging at 4ºC. They are stable for weeks at 4ºC.

For plasma and serum samples, blood should be cooled and centrifuged within one hour after blood collection. For serum preparation blood should spin at 3.000 rpm for 10 minutes, and the plasma samples, the conditions are 15 minutes at 3.500 rpm.
The plasma samples should be promptly separated from cells and transferred to a 1.5 ml clean tube, the intermediate layer include white blood cell, platelet not be transferred with the plasma.

For blood serum sample, blood is generally obtained using a plain (non-anticoagulanted) glass tube that permit the blood clot after the serum is harvested and transferred to a smaller tube for transport.
If extraction is not possible within three days , the plasma and serum should be immediately frozen to preferably -80ºC, but at least -20ºC.

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA
than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated
leukocytes; and the bottom layer contains concentrated erythrocytes.

3.2 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 **1.3 ml** (50 extractions) or in **2 x 3.35 ml** (250 extractions) 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 extractions) or **50 ml** (250 extractions) of Ethanol 100 % to the Desinhibition Buffer. 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 70ºC.

3.4 Protocol for genomic DNA extraction from whole blood (leukocytes)

This protocol is for the purification of genomic DNA from leukocytes by performing a selective lysis of the erythrocytes with the RBC Lysis Buffer. If only genomic or mitochondrial DNA is required, this is the ideal method because it produces better results in terms of quality and performance.

1. Pipet **300 µl of blood** into a 1.5 ml microtube. **Add 900 µl of RBC Lysis Buffer**. Vortex and incubate at room temperature for 10 minutes.

2. **Centrifuge at maximum speed for 1 minute**. Eliminate the supernatant by decanting better than with a micropipette that can aspirate the small non-visible cell pellet and leave 10-20 µl of residual liquid. Vortex the microtube to resuspend the pellet.

3. Add 1**80 µl of Lysis Tissue Buffer** + **25 µl Proteinase K.** Resuspend the cell pellet with micropipette.

4. **Incubate at 56ºC** for 15 minutes.

5. Add **200 µl of Lysis / Binding Buffer.** Mix by vortex. **Incubate at 70ºC for 10 minutes**.

6. Add **200 µl of Ethanol (96-100%).** Mix by vortex.

7. **Transfer the sample to a MicroSpin column with its collection tube.**

8. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample has not completely passed, repeat the centrifugation step.

9. Place the Spin column in a new collection tube and add **500 µl of Desinhibition Buffer** to the reservoir**. Centrifuge at 8.000 rpm for 60 seconds.** Remove the liquid.

10. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

11. 2º Wash. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. . Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

12. **Centrifuge at maximum speed for 2 minutes to remove the residual ethanol.**

13. Remove the collection tube and insert the Spin Column in a 1.5 ml microtube **. Add 50-200 µl of elution buffer** (preheated at 70ºC) into reservoir of Spin column. Dispense buffer directly onto the silica membrane. **Incubate at room temperature for 1 min**

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

3.5 Protocol for genomic DNA extraction from whole blood, buffy coat and biologics fluids

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge.

1. **Pipet 25 µl proteinase K** into the bottom of a 1.5 ml microcentrifuge tube.

2. **Add 300 µl sample** to the microcentrifuge tube. Use up to 300 µl whole blood, plasma, serum, buffy coat, or body fluids. For sample volumes less than 300 µL, add PBS to adjust the volume to 200 µL. If purifying DNA viruses, we recommend starting with 300 µL serum or plasma.

3. Add **300 µl of the Lysis/ Binding Buffer** of the sample material. Vortex the mixture vigorously (10–20 s). **Incubate at 70ºC for 15 minutes**. The lysate should become brownish during incubation. *Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.*

4. **Add 300 µL ethanol (96–100 %)** to each sample and vortex again.

5. Pipette the half of the lysate into reservoir of a combined Spin Column –collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.**

6. Repeat the point 5with the another half of the lysate.

7. Place the Spin column in a new collection tube and add **500 µl of Desinhibition Buffer** to the reservoir**. Centrifuge at 8.000 rpm for 60 seconds.** Remove the liquid.

8. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

9. 2º Wash. **Add 500 µl of Wash Buffer** into reservoir of Spin column**. . Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

10. **Centrifuge at maximum speed for 2 minutes to remove the residual ethanol.**

11. Remove the collection tube and insert the Spin Column in a 1.5 ml microtube **. Add 50-200 µl of elution buffer** (preheated at 70ºC) into reservoir of Spin column. Dispense buffer directly onto the silica membrane. **Incubate at room temperature for 1 min**

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

3.4 Protocol for genomic DNA extraction from dried blood spots

Sample sizing step
Place 3 punched-out circle from dried blood spot into 1.5 ml tube.
Pre-treating step
Add 200 µl PBS and vortex vigorously. Incubate at 85ºC for 10 minutes. Briefly centrifuge to remove drops from inside the lid.

1. **Pipet 25 µl proteinase K** into the sample.

2. Add **200 µl of the Lysis/ Binding Buffer** of the sample material. Vortex the mixture vigorously (10–20 s). **Incubate at 70ºC for 1 hour**.

3. **Add 200 µL ethanol (96–100 %)** to each sample and vortex again. To continue with the normal protocol at the point 5.

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 DANAGE-BIOTED S.L info@danagen.es