DANAGENE SPIN BLOOD DNA KIT

Ref. 0606.1 50 extractions
Ref. 0606.2 250 extractions

1. INTRODUCTION

This kit is designed for rapid extraction and purification of **high quality genomic DNA from whole blood, serum, plasma and biological fluids** using Spin columns with silica membrane that selectively binds DNA

This Kit uses a new Lysis/Union Buffer formulated specifically for the extraction of DNA from blood samples for high performance.

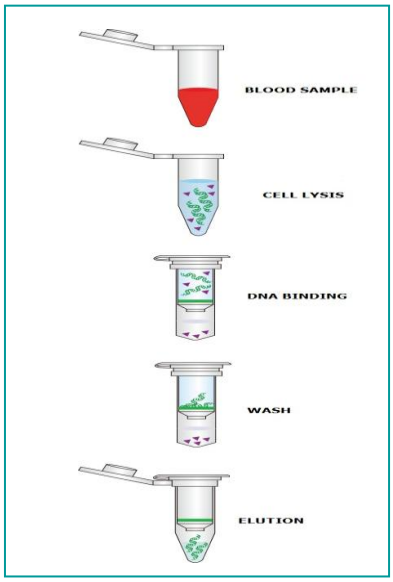
Characteristics:

- For rapid collection of DNA of high quality and ready for use from blood.
- Sample size: 300 µl of whole blood, plasma, serum and biological fluids.
- No organic extractions or precipitations with alcohol are used.
- Complete elimination of inhibitors or contaminants.
- Typical yield: 6-9 µg genomic DNA.
- Elution volume: 50-200 µl.
- A high quality DNA is obtained that can be used directly in PCR, Southern, cloning and in any enzymatic reaction.

Applications:

- Extraction of genomic, viral and bacterial DNA.
- DNA from whole blood (human or animal blood, fresh or frozen).
- DNA from blood treated with citrate, EDTA or heparin.
- DNA from serum, plasma, platelets, buffy coat, biological fluids and dried blood spots.

Procedure: In the case of blood samples and if genomic DNA is required, the best option is a previous and selective lysis of erythrocytes with the RBC Lysis Buffer to process only the lymphocytes that will provide us better results in quality and performance. If another type of DNA is being sought (bacterial, viral) lysis is achieved by incubating whole blood in a chaotropic solution in the presence of proteinase K at 70°C. The appropriate conditions will be created for the DNA to bind to the silica fiber membrane by adding ethanol to the lysate. The contaminants are removed by 2 different washes and finally the DNA is eluted with an elution buffer.



2. KIT INCLUDES

Reagents	Ref.0606.1 50 extractions	Ref.0606.2 250 extractions	Tª Store
RBC Lysis Buffer	50 ml	250 ml	15-25ºC
Tissue Lysis BUffer	10 ml	50 ml	15-25ºC
Lysis/Union 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
MicroSpin Columns	50 units	250 units	15-25ºC
Collection Tubes	100 units	500 units	15-25ºC

(*) These solutions should be prepared as indicated in the sections of the Protocol Preliminary Preparations.

Equipment and reagents required and not provided

- Etanol 100%.
- 1.5 ml microtubes.
- Microcentrifuge.
- Vortex.
- 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/Union Buffer and Desinhibition Buffer contain guanidine hydrochloride which is irritating, wear gloves and goggles.

3. PROTOCOL

3.1 Collection and storage of samples

Samples of whole blood should be stored at 4°C immediately after collection. They are stable for weeks at 4°C. They can also be shipped in refrigerated containers.

Plasma and serum samples should be cooled and centrifuged immediately within one hour of being obtained. For the preparation of blood serum centrifuge at 3,000 rpm for 10 minutes and for plasma samples centrifuge at 3500 rpm for 15 minutes.

Plasma samples should be separated from cells and passed to a new 1.5 ml microtube, the intermediate layer including white cells, platelets, should not be transferred with the plasma.

Serum samples are usually obtained with normal glass tubes without anticoagulants that allow the formation of blood clots after the serum is collected, and then passed to a new microtube for transport.

If extraction is not possible within three days of being obtained, plasma and serum should preferably be frozen at -80°C or at least -20°C.

Buffy coat is a fraction enriched in leucocytes from whole blood. Preparing this fraction from whole blood is simple and has a yield of about 5-10 times more DNA than an equivalent volume of whole blood. Its preparation is performed by centrifuging the blood at 2500xg for 10 minutes at room temperature. After centrifugation, 3 fractions are observed. The upper fraction is plasma; the intermediate is the buffy coat that contains the concentrated leukocytes; and the lower fraction contains erythrocytes.

3.2 PreLab Preparations

• Dissolve Proteinase K in **1.3 ml** (50 extractions) or **2 x 3.35 ml** (250 extractions) in nuclease-free water 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 Lysis/Union Buffer has no precipitates due to the low temperatures. If necessary, dissolve the precipitates by heating the solution to 37°C.

• Add the Ethanol 100% indicated on the label to the Desinhibition Buffer, about **10 ml** (50 extractions kit) and about **50 ml** (250 extractions kit). Keep the container tightly closed to avoid evaporation of ethanol.

• Add the Ethanol 100% indicated on the label to the Wash Buffer, about **40 ml** (kit 50 extractions) and about **200 ml** (kit 250 extractions). Keep the container tightly closed to avoid evaporation of ethanol.

• Preheat the Elution Buffer to 70°C.

3.3 Protocol for extraction of genomic DNA from blood lymphocytes

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

1. Pipette **300 µl of blood** into a 1.5 ml microtube. Add **900 µl of RBC Lysis Buffer**. Mix with vortex type stirrer and incubate at room temperature for 10 minutes.

2. **Centrifuge at full speed for 1 minute**. Eliminate the supernatant by decantation better than with a micropipette, since the small non-visible cellular pellet can be aspirated, leaving 10-20 µl residual liquid. Shake the microtube with a vortex-type stirrer to resuspend the pellet.

3. **Add 180 µl Tissue Lysis Buffer** and **25 µl Proteinase K**. Mix with vortex type stirrer for 2-5 seconds.

4. **Incubate at 56°C for 10 minutes**.

5. **Add 200 µl of Lysis/Union Buffer**. Mix with vortex type stirrer. **Incubate at 70°C for 10 minutes**.

6. **Add 200 µl of Ethanol** (96-100%) to the lysate. Mix vortex type stirrer.

7. **Pass the sample to a MicroSpin column** with your 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 MicroSpin column into a new collection tube and add **500 µl of Disinhibition Buffer** into the reservoir of the MicroSpin column. **Centrifuge at 12,000-14,000 rpm for 60 seconds**. Remove the liquid.

10. **Add 500 µl of Wash Buffer** to the MicroSpin Reservoir column. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

11. 2nd Washing. **Add 500 µl of Wash Buffer** to the reservoir of the Spin column. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

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

13. Remove the collection tube and insert the MicroSpin column into a 1.5 ml microtube. **Add 50-200µl of Elution Buffer** (preheated to 70°C) in the MicroSpin column reservoir. **Incubate 1 minute**.

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

3.4 Protocol for the extraction of DNA from whole blood, buffy coat and biological fluids

This protocol is for the purification of total DNA (genomic, mitochondrial, bacterial and viral) from whole blood, buffy coat and biological fluids.

1. **Pipette 25 µl proteinase K** into a 1.5 ml microtube.

2. **Add 300 µl of sample** to the microtube. Use up to 300 µl of whole blood, plasma, serum, buffy coat or biological fluid. For samples smaller than 300 µl, add PBS to adjust the volume to 300 µl. If we purify viral DNA, we recommend starting with 200 µl of serum or plasma.

3. **Add 300 µl of the Lysis/Union Buffer**. Shake the mixture vigorously with a vortex stirrer (for 10-20 s). **Incubate at 70ºC for 15 minutes**. *The lysate will turn brown during incubation. If old or coagulated blood is processed, increase the proteinase K incubation period to 30 minutes and shake the mixture vigorously with a vortex-type stirrer several times during the incubation process.*

4. **Add 300 µl ethanol** (96-100%) to each sample and mix with a vortex type stirrer.

5. Pipette and add half of the lysate into the reservoir of the MicroSpin column with its collection tube. **Centrifuge at 10,000 rpm for 60 seconds**. Remove the collection tube.

6. Repeat step 5 with the other half of the lysate.

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

8. **Add 500 µl of Wash Buffer** to the reservoir of the MicroSpin column. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

9. 2nd Washing. **Add 500 µl of Wash Buffer** to the reservoir of the MicroSpin column. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

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

11. Remove the collection tube and insert the spin column into a 1.5 ml microtube. **Add 50-200µl of Elution Buffer** (preheated to 70°C) in the MicroSpin column reservoir. **Incubate 1 minute**.

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

3.5 Protocol for extracting genomic DNA from dried blood spots

Step Obtaining Sample

Place 3 circles obtained from perforating a dried blood spot in a 1.5 ml microtube.

Pre-treatment step

Add 200 µl of PBS and mix vigorously with a vortex type stirrer. Incubate at 85°C for 10 minutes. Briefly centrifuge to remove the drops from the plug.

1. **Add 25 µl proteinase K** to the sample.

2. **Add 200 µl of the Lysis/Union Buffer**. Shake the mixture vigorously with a vortex stirrer (for 10-20 s).

3. **Incubate at 70°C for 1 hour**.

4. **Add 200 µl of ethanol** (96-100%) to each sample and stir the mixture with a vortex-type stirrer. Continue in section 5 of the protocol section 3.4.

4. GUIDE TO PROBLEMS AND SOLUTIONS

For any further questions or queries about the protocol, please contact DanaGen-BioTed Technical Service at info@bioted.es or info@danagen.es