DANAGENE BLOOD DNA KIT

Ref. 0601 100 ml
Ref. 0602 200 ml

1. INTRODUCTION

DANAGENE BLOOD DNA Kit provides a method for extracting high quality genomic DNA from whole blood or bone marrow.

It is a fast, safe and economical method. Its protocol is scalable allowing to process samples of different sizes. It uses a step of deproteinization with a novel salt buffer avoiding the use of toxic organic solvents like phenol or chloroform.

This kit provides sufficient reagents for the processing of 100-200 ml -depending on the size of the kit- of blood.

DNA uptake ranges from 15-45 µg/ml of whole blood and is free of PCR inhibitors and other enzymatic reactions.

2. KIT INCLUDES

	Ref. 0601 100 ml	Ref. 0602 200 ml	Store
RBC lysis solution	300 ml	600 ml	Room temperature
Lysis solution	100 ml	200 ml	Room temperature
Protein precipitation solution	35 ml	70 ml	Room temperature
Hydration solution	35 ml	70 ml	Room temperature

Equipment and reagents required and not provided

- Isopropanol.
- Ethanol 70%.
- 1.5 ml microtubes, 15 or 50 ml centrifuge tubes.
- Microcentrifuge or clinical centrifuge.
- 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.

3. PROTOCOL

The protocol involves the following steps:

- **Selective lysis of erythrocytes**, cells containing DNA are separated from erythrocytes that are lysed.

- **Cell lysis**. The cells are lysed with an anionic detergent that solubilizes cellular components.

- **Proteins precipitation**. Cytoplasmic and nuclear proteins are removed by saline precipitation.

- **DNA precipitation**. Genomic DNA is extracted with precipitation with isopropanol.

- **DNA hydration**. The DNA pellet is dissolved in sterile water or the hydration solution by incubation at 65°C or overnight at room temperature and shaking.

3.1 PreLab Preparations

If the Lysis Solution contains a precipitate due to the low temperatures, incubate at 37 ° C and mix to dissolve the precipitate.

3.2 Protocol extraction from whole blood

1. Collect blood in tubes containing 15% EDTA to reduce DNA degradation and coagulation, other anticoagulants such as sodium citrate or heparin also work well.

2. Fresh samples can be stored at 4°C for no more than 5 days.

3. Frozen samples are stable at -80°C for two years.

Extraction from 300 µl samples. Microtubes 1.5 - 2.0 ml and microcentrifuge.

Cell Lysis

1. Add 300 µl of blood into a microtube containing 900 µl of **RBC lysis solution**. Mix and incubate for 10 minutes at room temperature. Invert the tube several times during incubation.

2. Centrifuge for 20-30 seconds at 13000-16000 x g. Remove the supernatant with pipette without damaging the visible cell pellet and leave between 10-20 µl of residual liquid.

3. Vortex the microtube to resuspend the pellet, which will help the cell lysis from step 4.

4. Add 300 µl of **Lysis solution** and resuspend by pipette to lyse the cells.

It is very important to observe the homogeneous solution without cell groups, it is recommended to incubate at 37ºC for 5 minutes or until the homogeneous solution is observed.

Protein precipitation.

OPTIONAL STEP: Add 1.5 µl RNase (10 mg/ml) and mix well. Incubate for 15 minutes at 37°C.

1. Cool the sample to room temperature.

2. Add 100 µl of **Protein precipitation solution** to the cell lysate. You have to vortex the mix vigorously at full speed for 20-30 seconds.

3. Centrifuge at 13000-16000 x g for 3-5 minutes. A dark brown precipitate will form. If floating particles are observed, centrifuge again after incubating 5 minutes on ice.

DNA precipitation.

1. Transfer the supernatant containing the DNA to a fresh microtube containing 300 µl of **Isopropanol**.

2. Mix by inversion about 50 times.

3. Centrifuge at 13000-16000 x g for 2 minutes. The DNA will be visible as a white pellet.

4. Remove the supernatant and dry the tube briefly on absorbent paper. Add 300 µl of **Ethanol 70%** to wash the DNA.

5. Centrifuge at 13000-16000 x g for 1 minute. Carefully remove the supernatant without touching the DNA pellet. It can be centrifuged briefly again to collect the last drops of residual ethanol with a micropipette, avoiding touching the DNA pellet.

6. Invert the microtube over an absorbent paper and let it dry for about 15 minutes.

DNA hydration.

1. Add between 100 µl of the **Hydration solution** and resuspend with a pipette.

2. Store at 2-8°C. For long storage, store at -20°C or -80°C.

Extraction from 3 ml samples. Tubes 15 or 50 ml and clinical centrifuges.

Cell Lysis

1. Add 3 ml of blood into a tube containing 9 ml of **RBC lysis solution**. Mix and incubate for 10 minutes at room temperature. Invert the tube several times during incubation.

2. Centrifuge for 10 minutes at 2000 x g. Remove the supernatant with a pipette without damaging the visible cell pellet and leave between 100-200 µl of residual liquid.

3. Vortex the tube to resuspend the pellet, which will assist cell lysis step 4.

4. Add 3 ml of **Lysis solution** and resuspend by pipette to lyse the cells.

It is very important to observe the homogeneous solution without cell groups to increase the DNA yield, it is recommended to incubate at 37ºC for 5 minutes or until the homogeneous solution is observed.

Protein precipitation.

OPTIONAL STEP: Add 15 µl RNase (10 mg/ml) and mix well. Incubate for 15 minutes at 37°C.

1. Cool the sample to room temperature.

2. Add 1 mll of **Protein precipitation solution** to the cell lysate. You have to vortex the mix vigorously at full speed for 20-30 seconds.

3. Centrifuge at 2000 x g for 5 minutes. A dark brown precipitate will form. If floating particles are observed, centrifuge again after incubating 5 minutes on ice.

DNA precipitation.

1. Transfer the supernatant containing the DNA to a fresh tube containing 3 ml of **Isopropanol**.

2. Mix by inversion about 50 times.

3. Centrifuge at 2000 x g for 3 minutes. The DNA will be visible as a white pellet.

4. Remove the supernatant and dry the tube briefly on absorbent paper. Add 3 ml of **Ethanol 70%** to wash the DNA.

5. Centrifuge at 2000 x g for 2 minute. Carefully remove the supernatant without touching the DNA pellet. It can be centrifuged briefly again to collect the last drops of residual ethanol with a micropipette, avoiding touching the DNA pellet.

6. Invert the microtube over an absorbent paper and let it dry for about 15 minutes.

DNA hydration.

1. Add between 250-500 µl of the **Hydration solution** and resuspend with a pipette.

2. Incubate at 65 ° C for 1 hour with periodic stirring to aid DNA dispersion, or incubate overnight at room temperature with gentle shaking.

3. Transfer to a 1.5 ml microtube and store at 2-8°C. For long storage, store at -20°C or -80°C.

Table of volumes of reagents scaled from 5 µl to 10 ml

Blood volume	5-25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
Tube size	1.5 ml	1.5 ml	1.5 ml	2.0 ml	15 ml	50 ml	50 ml
RBC lysis solution	75 µl	150 µl	600 µl	1.5 ml	3 ml	15 ml	30 ml
Lysis solution	5-25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
Precipitation solution	5-10 µl	17 µl	67 µl	170 µl	330 µl	1.67 ml	3.3 ml
Isopropanol	25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
Ethanol 70%	25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
µg DNA obtained	0.15-0.75	0.8-2.0	3.0-8.0	7.0-23	15-40	75-200	150-400

4. GUIDE TO PROBLEMS AND POSSIBLE SOLUTIONS

1. **Incomplete lysis of erythrocytes**.
Re-incubate with **RBC Lysis solution**.

2. **Presence of blood clots in the whole blood sample**.
The sample was not properly preserved or improperly mixed in EDTA in the collection tube. Extract the DNA only from the uncoagulated sample portion, avoiding transferring clots from the collection tube.

3. **Incomplete cell lysis.**
3.1 Because the number of cells was too large for the amount of **Lysis solution** used. To solve it add more amount of the **Lysis solution**.
3.2 Due to the formation of clumps or clusters of cells, this occurs when cells are not resuspended correctly before the **Lysis solution** is added. Incubate in the **Lysis solution** until the solution is homogeneous.

4. No protein precipitation occurs.
4.1 The sample was not sufficiently cooled prior to the addition of the **Protein precipitation solution**.
4.2 Not sufficiently mixed with **Protein precipitation solution**. You have the mixer the solution with vortex the indicated time.
4.3 Spin speed was not correct. For microcentrifuges use the maximum speed. For other centrifuges the speed is 2000 x g which is not equivalent to 2000 rpm. If your centrifuge does not reach 2000 x g increase the centrifugation time.

5. Low rehydration of DNA.
5.1 Samples were not mixed during the rehydration step. Mix samples periodically.
5.2 The pellets were dried to excess. Increase the rehydration time. Do not incubate overnight at 65°C.

6. Protein contamination in rehydrated DNA.
Because was used a sample too large. Re-extract the DNA.

7. DNA quality.
The $A_{260}/_{280}$ is too high or too low. If the sample is contaminated with proteins we will have a value <1.6. By contrast, if is greater than 2.0, the sample will contain RNA, perform an RNAse treatment. Although we have poor values, the best indicator of DNA quality is whether the extracted DNA can be digested with restriction enzymes or amplified using PCR.

8. The obtained DNA is less than 50Kb size.
The DNA is degraded due to incorrect collection of the sample or incorrect storage of the material to be used.

9. Low DNA uptake.
9.1 Cell lysis was not complete. This step is very important; the lysis must be prolonged until the sample is homogeneous.
9.2 Presence of clumps or groups of undissolved cells after addition of the **Lysis solution**. It is very important, to dissolve these groups of cells, can be avoided by resuspension of the cellular pellet prior to the addition of the **Lysis solution**.

For any further questions or queries, please contact us at info@danagen.es