



DANAGENE BLOOD DNA KIT

Ref. 0601 100 ml

Ref. 0602 200 ml

1. INTRODUCTION

The DANAGENE BLOOD DNA Kit is a method for the extraction of high quality genomic DNA from mammalian whole blood or bone marrow.

It is a fast, save and economical method. Its protocol can be scale allowing to process different size samples. The protocol includes a deproteination step using a new saline buffer, avoiding the use of toxic organic solvents such as phenol or chlorophorm.

This Kit provides enough reagents to process 100-200 ml (depending on ref.) of blood.

The DNA extraction oscillates between 15-45 μg / ml of blood and it is free from PCR inhibitors and other enzymatic reactions.

2. KIT COMPONENTS

	Ref. 0601 100 ml	Ref. 0602 200 ml	
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
Hydratation Solution	35 ml	70 ml	Room temperature

Materials to be supplied by the user

- * Isopropanol.
- * 70% Ethanol.
- * 1.5 ml microtubes, 15 or 50 ml centrifuge tubes.
- * Microcentrifuge or clinique centrifuge.
- * Vortex.
- * Water bath.

3.PROTOCOL

The protocol implies the following steps:

1. **Erythrocyte selective lysis.** Cells containing DNA are isolated from the erythrocytes, which are lysed.
2. **Cell lysis.** The cells are lysed with an anionic detergent that solubilizes the cell components.
3. **Protein precipitation.** Proteins and cellular debris are removed by precipitation .
4. **DNA precipitation.** The genomic DNA is obtained by a precipitation with isopropanol.
5. **DNA hydration.** The DNA pellet is dissolved in sterile water or Hidratation Solution by short incubation at 65°C or keeps it mixing at room temperature overnight. The hydratation with TE allows the DNA conservation for a long time.

3.1 Preliminary considerations

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

3.2 EXTRACTION FROM BLOOD PROTOCOL

1. Collect blood in tubes containing 15 % EDTA to reduce the DNA degradation and blood coagulation, anticoagulants such as sodium citrate or heparin can also be used.
2. Fresh samples can be stored at 4°C for a maximum of 5 days.
3. Frozen samples are stable at -80°C for two years.

EXTRACTION FROM 300 µl SAMPLES.

(Using 1.5 - 2.0 ml microtubes and microcentrifuge)

✿ Cell Lysis

1. Add 300 µl of whole blood (or bone marrow) to a microtube containing 900 µl of **RBC Lysis Solution** . Mix and incubate for 10 minutes at room T^a. Turn the tube upside down several times during the incubation period.
2. Centrifuge for 20-30 seconds at 13.000-16.000 x g. Remove the supernatant using a pipette and avoiding damaging the cell visible pellet and leaving 10-20 ml of residual liquid.
3. Vortex the microtube to resuspend the pellet, this process will help to optimize the cell lysis in the following step.
4. Add 300 µl of **Lysis Solution** and resuspend with pipette to lyse the cells. It is very important to observe that the solution is homogeneous without groups of cells , it is recommended to incubate at 37°C for 5 minutes or until the solution is homogeneous.

✿ Protein precipitation.

1. Cool sample to room temperature.
2. Add 100 µl of **Protein precipitation solution** to the cell lysate.
3. Vortex vigorously at maximum speed for 20-30 seconds.
4. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A dark brown precipitate will be formed. If there are particles floating in the supernatant centrifuge again once you have incubated in ice for 5 minutes.

✿ DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing 300 µl of **Isopropanol**.
2. Mix turning the tube upside down for about 50 times.
3. Centrifuge at 13.000-16.000 x g for 2 minutes. The DNA will be visible as a white pellet.
4. Remove the supernatant and dry the tube on absorbent paper. Add 300 µl of **Ethanol 70%** to wash the DNA.
5. Centrifuge at 13.000-16.000 xg for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.
6. Turn the microtube upside down on absorbent paper and leave it to dry for about 15 minutes.

✱ **DNA Hydration**

1. Add 100 µl of sterile water or TE and mix well with pipette.
2. Keep it at 2-8°C. To storage for a long time it is recommended to dissolve it with TE and to keep it at -20°C/ -80°C.

EXTRACTION FROM 3 ml SAMPLES
(Using Tubes of 15 or 50 ml and clinic centrifuges)

✱ **Cell Lysis**

1. Add 3 ml of whole blood (or bone marrow) to a tube containing 9 ml of **RBC Lysis Solution** . Mix and incubate for 10 minutes at room T^a. Turn upside down the tube several times during the incubation.
2. Centrifuge for 10 minutes at 2.000 xg. Remove the supernatant with a pipette avoiding damaging the visible cell pellet and leaving 100-200 µl of residual liquid.
3. Vortex the tube to resuspend the pellet, this process will help to optimize the cell lysis in the following step.
4. Add 3 ml of **Lysis Solution** and resuspend with pipette to lyse the cells. It is very important to obtain a homogeneous solution without groups of cells, it is recommended to incubate at 37°C for 5 minutes until you obtain a homogeneous solution.

✱ **Protein precipitation.**

1. Cool sample to room temperature.
2. Add 1.0 ml of **Protein precipitation solution** to the cell lysate.
3. Vortex vigorously at maximum speed for 20-30 seconds.
4. Centrifuge at 2.000 x g for 5 minutes. A dark brown precipitate will be formed. If there are particles floating, centrifuge again once you have incubated 5 for minutes in ice.

✱ **DNA precipitation.**

1. Transfer the supernatant, which contains the DNA, into a new tube containing 3 ml of **Isopropanol**.
2. Mix turning the tube upside down for about 50 times.
3. Centrifuge at 2.000 x g for 3 minutes. The DNA will be visible as a white pellet.
4. Remove the supernatant and dry briefly the tube on absorbent paper. Add 3 ml of **70%Ethanol** for washing the DNA.
5. Centrifuge at 2.000 x g for 2 minutes. Remove carefully the supernatant avoiding touching the pellet of DNA. It can be briefly centrifuge the last drops of residual ethanol.
6. Turn the tube upside down on an absorbent paper and let it to dry for about 15 minutes.

✱ **DNA hydration.**

1. Add 250-500 µl of sterile water or TE and dissolve with pipette.
2. Incubate at 65°C for 1 hour shaking periodically to help DNA dispersion, or incubate overnight at room temperature shaking gently.
3. Transfer to a micro tube of 1.5 ml and keep it at 2-8°C. To storage for a long time it is recommended to mix well with TE and to keep it at -20°C or - 80 °C.

SCALED REACTIVES VOLUMES TABLE 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 sol.	75 µl	150 µl	600 µl	1.5 ml	3 ml	15 ml	30 ml
Lysis sol.	5-25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
Precipitation sol.	5-10 µl	17 µl	67 µl	170 µl	330 µl	1.67ml	3.3 ml
Isopropanol	25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
70% Etanol	25 µl	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
mg of DNA obtained	0.15-0.75	0.8-2.0	3.0-8.0	7.0-23	15-40	75-200	150-400

4. PROBLEM GUIDE AND POSSIBLE ANSWER

1. *Erythrocyte incomplete lysis.*

Incubate once again with the RBC Lysis Solution.

2. *Presence of blood coagulum's in the blood sample.*

The sample was not kept in a correct way or was mixed up with the EDTA in the collecting tube in a wrong way. Extract DNA only from the non-coagulated portion of blood, avoiding transferring coagulum from the collecting tube.

3. *Incomplete cell lysis.*

3.1 Due to the fact that the number of cells was too big for the Lysis solution quantity used. Add more Lysis solution.

3.2 Due to the formation of groups of cells, this happens when the cells are not correctly dissolved before adding the Lysis Solution. Incubate in the Lysis solution until the solution is homogeneous.

4. *The protein precipitation is not produced.*

4.1 The sample was not cooled enough before the addition of the Precipitation Solution.

4.2 It was not mixed up enough with the Precipitation Solution. Shake with vortex for the indicated time.

4.3 The centrifugation speed was not correct. For micro centrifuges use the maximum speed. For other centrifuge the speed must be of 2.000 x g, which is not equivalent to 2.000 r.p.m. If your centrifuge does not reach 2.000 x g increase the centrifugation time.

5. *Low DNA rehydrated.*

5.1 The samples were not mixed up during the rehydrated step. Mix up the samples periodically.

5.2 The pellets were dried in excess. Increase the rehydrating period. Do not incubate over night at 65°C.

6. *Protein contamination of the rehydrated DNA.*

Due to the fact that the sample used was too big. Re-extract the DNA.

7. *DNA quality.*

The relation $A_{260/280}$ obtained is too high or too low. If the sample is contaminated with proteins, the value obtained in the $A_{260/280}$ will be less than 1.6. Unlike, if the obtained ratio is higher than 2.0, it indicates that the sample contains RNA, for removing it, it is recommended to make a treatment with RNase.

Another indicator of the extracted DNA quality is the fact that it can be digested with restriction enzymes or amplified with PCR techniques.

8. *The DNA obtained is less than 50Kb.*

The DNA is degraded due to an incorrect sample collection or an incorrect storage of the material that is going to be used.

9. *Low DNA obtaining.*

9.1 The cell lysis was incomplete. This is a very important step, the lysis must continue until the sample is homogeneous.

9.2 Presence of groups of cells that have not been dissolved after the addition of the Lysis solution. It is very important to dissolve these groups of cells before the addition of the Lysis Solution.