DANAGENE TURBO BLOOD KIT

Ref. 1104 100 Preps

1.INTRODUCTION

This kit allows the obtention of **PCR- ready genomic DNA from whole blood or semen samples** in just 5 minutes.

The protocol consists in the incubation in the lysis solution of 5 ml of whole blood for 5 minutes, allowing the DNA liberation, after this a neutralization solution is added, the obtained extract can be directly used for doing PCR amplifications.

The kit, apart from the reagents for the extraction, includes ready to use- **HOT STAR Polimerase 2x ,** which allows the amplification of any fragment from the extract in a way that the consuser only has to add water and the primers. It requires a 7 minutes activation step at 95ºC in order to remove the non- specifics products such as primers-dimers and mis-primed products. It also contains a red dye which permits easy visualization and direct loading onto a gel avoiding the need of mixing with a loading buffer.

2. KIT COMPONENTS

	100 Preps	Storage
Lysis Solution	2.5 ml	4ºC
Neutralization Solution	18 ml	4ºC
"HOT STAR" Polimerase	1.25 ml	-20ºC

The HOT STAR polimerase can be stored at 4ºC for 2 weeks, for long-term storage store at -20ºC.

Equipment and additional reagents required

- Nuclease-free water
- Primers for the PCR
- Microtubes or PCR plates
- Microcentrifuge
- Thermal cycler

3.PROTOCOL

3.1 DNA extraction from blood

1. Collect the blood in tubes containing EDTA or sodium citrate. Mix well by inversion.
2. Put **5 µl of whole blood** in a microtube.
3. Add **20 µl of Lysis Solution.** Mix well with the pipette up and down.
4. Incubate at room temperature for 5 minutes.
5. Add **180 µl of Neutralization Solution.** Mix well with the pipette up and down.
6. Store the neutralized extract at 4ºC or use **2.5 µl** immediately in the PCR process. The DNA is stable at 4ºC for 4 months.

3.2 DNA extraction from blood collected in Matrix Paper

1. Collect the blood puncturing the finger with the lancet and place some drop some blood drops in the 5 circles drawn in the Matrix Paper. Allow air dry at room temperature. The Matrix Paper can be sent to the laboratory at room temperature, once there store at 4ºC.
2. Cut a square disc of **3 mm that contains blood** of the Matrix Paper and place it in a PCR microtube.
3. Add **25 µl of Lysis Solution** in such a way that the Matrix Paper is submerged in solution and mix well with the pipette up and down. The samples can be briefly centrifuged to collect the solution and the Matrix Paper.
4. Incubate in the thermalcycler at 75ºC for 5 minutes.
5. Add **180 µl of Neutralization Solution.** Mix well with the pipette up and down.
6. Store the neutralized extract at 4ºC or use **2.5 µl** immediately in the PCR process. The DNA is stable at 4ºC for 4 months.

3.3 DNA extraction from semen

1. Put 5 µl of semen into a PCR tube.
2. Add **20 µl of Lysis Solution.** Mix well with the pipette up and down.
3. Incubate into thermal cycler at 75ºC for 30 minutes.
4. Add **180 µl of Neutralization Solution**. Mix well with the pipette up and down.
5. Store the neutralized extract at 4ºC or use **2.5 µl** immediately in the PCR process. The DNA is stable at 4ºC for 4 months.

4 PCR amplification

1. Use **2.5 µl of the neutralized extract** for each PCR reaction.
2. The typical concentrations of the primers and cycling parameters will depend on the system used. A typical final concentration of the primers is 0.5 µM.

REAGENTS	**VOLUME**
Nuclease-free water	X µl
HOT STAR polimerase	12. 5 µl
Primers	Y µl
Neutralized extract	2.5 µl
Total Volume	**25 µl**

3. Mix well, the red stain included in the polimerase makes the process easier.
4. For those thermal cyclers without a heated lid, add 25 µl of mineral oil to prevent the evaporation.
5. Make the amplification process. The parameters of the amplification must be optimized for each pair of primers and DNA template.
6. **IMPORTANT: For activation of the HOT STAR polimerase it is necessary to program a denaturing initial step of 10 minutes at 95ºC,** after this, program the specific cycles of each product that´s going to be amplified.
7. The PCR product can be directly loaded in an agarose gel after the PCR, as the red stain acts as a loading buffer.

5 PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es