# DANAGENE TURBO TISSUES & CELL KIT

**Ref.1105**

## 1.INTRODUCTION

This kit allows the obtention of PCR**-ready genomic DNA** from tissue and animal samples, mouse tails, hair shafts and bacteria, in just **2 steps taking only 15 minutes.**

The DNA preparation can be laborious and take a long time, these methods can be necessary for some specific applications or if a big quantity of DNA is needed. In case of the PCR, a small quantity of DNA is needed and a high purity DNA is not required.

The DANAGENE Turbo Tissues and Cells Kit includes all the necessary reagents to extract PCR-ready DNA from small samples and amplify the target fragments by PCR.

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** | 10 ml | 4ºC |
| **Neutralization Solution** | 10 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 tissue

1. Add **100 µl of Lysis Solution** into a **PCR** microtube.
2. Put 2-10 mg of tissue in the solution. Mix well with vortex or pipette up and down, ensure that the tissue is submerged in the solution.
3. Incubate in the thermal cycler at 95ºC for 15 minutes. Tissues will not be completely digested at the end of the incubations, this is normal and will not affect performance.
4. Add **100 µl of Neutralization Solution** and mix by vortex.
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.

### 3.2 DNA extraction from mouse tail

1. Add **100 µl of Lysis Solution** into a PCR microtube.
2. Place 0.5-1.0 cm of the tipe of a mouse tail into solution. Mix well with vortex, ensure that the tail is well submerged in the solution.
3. Incubate in the thermal cycler at 95ºC for 15 minutes. Tissues will not be completely digested at the end of the incubations, this is normal and will not affect performance.
4. Add **100 µl of Neutralization Solution** and mix by vortex.
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.

### 3.3 DNA extraction from hair shafts

1. Add **100 µl of Lysis Solution** into a PCR microtube.
2. Place 1 or 2 hair shafts into the solution. Trim excess off of the hair shaft leaving the root Mix well with vortex or pipette up and down, ensure that the hair is submerged in the solution.
3. Incubate in the thermal cycler for 15 minutes at 95ºC. Tissues will not be completely digested at the end of the incubations, this is normal and will not affect performance.
4. Add **100 µl of Neutralization Solution** and mix by vortex.
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.

### 3.4 DNA extraction from mamalian cells

1. Pellet by centrifugation.
2. Add **100 µl of Lysis Solution**. Mix well. Seal the microplate to prevent loss by evaporation during incubation.
3. Incubate in the thermal cycler at 95ºC for15 minutes.
4. Add **100 µl of Neutralization Solution** and mix by vortexing..
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