DANAGENE FFPE DNA KIT

Ref. 0610.1 50 extractions

1. INTRODUCTION

This kit is optimized to be a rapid method for isolating **DNA from paraffin-embedded formalin-fixed (FFPE) tissue samples.**

The procedure omits the use of xylene or d-limonene, flammable and malodorous compounds that are commonly used for dewaxing, a **DEPARAFINIZATION SOLUTION** with its own formulation is used for a complete dissolution of paraffin that will allow us to release the tissue.

Characteristics:

- Uses MicroSpin technology columns with silica membranes, with a special design that allows small elution volumes.
- Easy removal of paraffin.
- Safe method that avoids the use of xylene or other toxicants.
- Complete elimination of contaminants and inhibitors.
- Elution volume: 25-30 µl.
- DNA quality is optimal for applications such as quantitative PCR or Mass sequencing.

Applications:

- Rapid extraction of DNA from paraffin-embedded formalin-fixed (FFPE) specimens.
- DNA extraction from fresh or archived FFPE samples.
- Sample extraction on slides.
- Typical subsequent applications: PCR, quantitative PCR, STR analysis, Mass sequencing.

2. KIT INCLUDES

	50 extractions	Store
Dewaxing solution	**30 ml**	Room temperature
Tissue Lysis Buffer	**10 ml**	Room temperature
Lysis/Binding Buffer	**15 ml**	Room temperature
Proteinase K*	**65 mg**	-20ºC
Disinhibition Buffer*	**18 ml**	Room temperature
Wash Buffer*	**10 ml**	Room temperature
Elution Buffer	**10 ml**	Room temperature
MicroSpin Columns	**50 units**	Room temperature
Collection Tubes	**100 units**	Room temperature

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

3. PROTOCOL

3.1 PreLab Preparations

• Dissolve **Proteinase K** in **3.10 ml** of 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.
• **Both Lysis/Binding Buffer and Desinhibition Buffer** contain Guanidine hydrochloride which is an irritant. For this reason, we recommend the use of glasses and gloves for handling.
• Add **10 ml Ethanol 100%** to the **Disinhibition Buffer** indicated on the label. Keep container tightly closed to prevent evaporation of ethanol.
• Add **40 ml of Ethanol 100%** to the **Wash Buffer** indicated on the label. Keep container tightly closed to prevent evaporation of ethanol.
• Preheat the **Elution Buffer** to 70°C.

3.2 Protocol for extraction of genomic DNA from FFPE samples

DEPARAFINIZATION OF THE SAMPLE

1. To a section of 10 µm with a maximum of 15 mg of paraffin add **400 µl dewaxing solution** and vortex for 10 seconds.
2. Incubate at 60°C for 3 minutes to promote the dissolution of the paraffin.
3. Immediately vortex the sample vigorously at 60°C to dissolve the paraffin.
4. Centrifuge at maximum speed for 3 minutes to collect tissue.
5. Remove the supernatant with a pipette, avoid touching the precipitated tissue.
6. Add 1 ml of **ethanol** 100%. You have to vortex the samples for 20 seconds.
7. Centrifuge at full speed for 3 minutes. Remove the **ethanol** with a pipette, avoiding touching the tissue.
8. Place the open tubes at 55°C for 10 minutes to evaporate the **ethanol**.

DNA EXTRACTION

1. Add **200 µl of Tissue Lysis Buffer + 40 µl Proteinase K**. Mix with vortex for 2-5 seconds.

2. **Incubate at 55°C** until lysis is complete or overnight (if possible with shaking 600 rpm). Centrifuge briefly the microtube to collect the drops from the walls.

3. Add **20 µl Proteinase K and incubate 1-2 h at 55°C**. After this incubation there should be no visible tissue particles. Centrifuge the microtube to remove any remaining tissue particles in the solution. Transfer the supernatant to a new microtube.

4. **Incubate at 90°C for 1 hour**. This incubation will partially reduce the formaldehyde modifications of the nucleic acids. *If only one bath is available, place the sample at room temperature until the bath has reached 90°C.*

5. **Centrifuge briefly** to collect the drops that are formed on the wall of the microtube.

6. Add **300 µl of Lysis/Binding Buffer**. Mix with vortex. **Incubate at room temperature for 5-10 minutes**. *Allow the lysate to cool to room temperature*.

7. Add **100 µl of Isopropanol to the lysate**. Mix with vortex. Briefly centrifuge to collect the possible drops present in walls of the microtube.

8. **Pass the sample to a MicroSpin column** with your collection tube.

9. **Centrifuge at 8,000 rpm for 60 seconds**. Remove the collection tube. If the sample has not completely passed, repeat the centrifugation step.

10. Place the MicroSpin column into a new collection tube and add **500µl of Disinhibition Buffer**.

11. **Centrifuge at 12000-14000 rpm for 60 seconds**. Remove the liquid.

12. **Add 500 µl of Wash Buffer**.

13. **Centrifuge at 12000-14000 rpm for 60 seconds**. Remove the liquid.

14. **Centrifuge at maximum speed for 3 minutes to remove residual ethanol**.

15. Place the MicroSpin column in a 1.5 ml microtube and add 25-30 µl of **Elution Buffer** (5mM Tris.HCl, pH 8.5) **preheated to 70°C**. *Ensure that the **Elution Buffer** is dispensed directly into the center of the membrane for complete elution of bound DNA.*

16. **Incubate for 2 minutes**.

17. **Centrifuge at full speed for 1 minute**. The microtube now contains the genomic DNA.

4. GUIDE TO PROBLEMS AND SOLUTIONS

We recommend contacting DanaGen-BioTed technical support for any additional questions regarding work protocols or problems that may arise during work.

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