DANAGENE FFPE DNA KIT

Ref. 0610.1 50 isolations

1. INTRODUCTION

This kit is optimized for a fast method to isolate **DNA from formalin-fixed, paraffin-embedded (FFPE) tissue specimen.**

The procedure omits the use of flammable and malodorous xylene or d-limonene commonly used for deparaffinization, **a proprietary buffer formulation DEPARAFFINIZATION SOLUTION** is used for the complete dissolution of the wax to release the tissue.

Features:

- **Silica membrane technology with specials MicroSpin columns.**
- **Very easy paraffin removal.**
- **Safe method avoids xylene and other toxic.**
- **Complete removal of contaminants and inhibitors for reliable downstream applications.**
- **Low elution volume: 15-30 µl.**
- **The quality of DNA is suitable for the following applications as quantitative PCR or Next generation sequencing (NGS).**

Applications:

- **Rapid isolation of DNA from formalin-fixed, paraffin-embedded samples.**
- **Isolation of DNA from fresh and archived FFPE samples**
- **Isolation of DNA from specimen of object slides**
- **Typical downstream application: PCR, pPCR, NGS, STR analysis.**

2. COMPONENTS KIT

	50 isolations	Store
Deparaffinization solution	30 ml	Room Temp
Tissue Lysis Buffer	10 ml	Room Temp
Lysis/ Binding Buffer	15 ml	Room Temp
Proteinase K (*)	65 mg	-20ºC
Desinhibition Buffer (*)	18 ml	Room Temp
Wash Buffer (*)	10 ml	Room Temp
Elution Buffer	10 ml	Room Temp
MicroSpin Columns	50 units	Room Temp
Collection tubes	100 units	Room Temp

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol

3. PROTOCOL

3.1 Preliminary Preparations

- Both the Lysis/ Binding Buffer and the Desinhibition Buffer contain Guanidine hydrochloride which is an irritant agent , for this reason we recommend to use gloves and glasses for its manipulation.
- Dissolve the proteinase K in **3.10 ml** of nuclease-free water and store at –20ºC. It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.
- Add **10 ml** of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation
- Add **40 ml** of Ethanol 100 % to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.
- Pre-heat the Elution Buffer at 70ºC.

3.2 Protocol for genomic DNA extraction from FFPE samples para la

DEPARAFFINIZATION SAMPLE

1. Add **400 µl of Deparaffinization solution** to one FFPE tissue slice (10mm). *Make sure the sample does not comprise more than 15 mg paraffin.* **Vortex for 10 seconds.**

2. Incubate **3 minutes at 60ºC** to promote the melting of the paraffin.

3. Vortex the sample immediately (at 60ºC) at a vigorous speed to dissolve the paraffin. After 3 minutes you will see the tissue floating in the wax remover.

4. **Centrifuge full speed for 3 minutes** to pellet the tissue.

5. **Remove the supernatant** with pipetting, avoiding the tissue.

6. **Add 1 ml ethanol 100%.** Vortex for 20 seconds.

7. **Centrifuge full speed for 3 minutes** to pellet the tissue. Remove the ethanol with pipetting, avoiding the tissue.

8. Place the tubes **at 55ºC for 10 minutes** with caps open to evaporate the ethanol.

DNA ISOLATION

1. Add **200 μl of the Tissue Lysis Buffer** + **40 μl Proteinase K.** Mix by vortexing 20 seconds.

2. **Incubate at 55ºC** for 1 hour or until the lysis is complete (if possible with agitation 600 rpm). Centrifuge briefly to collect the drops.

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 to remove any remaining tissue that may remain. Transfer the supernatant to a new microtube.

4. **Incubate at 90ºC for 1 hour.** This incubation partially reverses formaldehyde modification of nucleics acids. *If using only one heating block, leave the sample at room temperature after the 55ºC incubation until the heating block has reached 90ºC.*

5. **Briefly centrifuge** to remove drops from the inside of the lid.

6. Add **300 μl of Lysis/Binding Buffer.** Mix by vortexing. **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 by vortexing.Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

8. **Transfer the lysate** into reservoir of a combined MicroSpin Column – collection tube.

9. **Centrifuge at 8.000 rpm for 60 seconds.** Remove the collection tube. If the sample is not drawn completely through the matrix, repeat the centrifugation step.

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

11. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

12. **Add 500 μl of Wash Buffer** into reservoir of MicroSpin column.

13. **Centrifuge at 12.000-14.000 rpm for 60 seconds.** Remove the liquid.

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

15. Remove the collection tube and insert the MicrSospin column in a 1.5 ml microtube**. Add 25- 30 µl of Elution Buffer** (preheated at 70ºC) directly onto the center of the silica membrane.

16. **Incubate 2 minutes.**

17. **Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

4. 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