canvax

DATA SHEET

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1. Identification

Product name	**HigherPurity™ FFPE DNA Isolation Kit**
Cat. No	**AN0160 (50 reactions)**
Cat. No	**AN0160-XL (250 reactions)**

2. Description

Formalin fixation and paraffin embedding (FFPE) is a standard method for long-term preservation of most archived pathological specimens. Such samples provide an invaluable resource for subsequent molecular studies of clinical phenotypes, especially genetic studies in which DNA is not available from fresh or frozen tissues. FFPE tissue is an excellent source of DNA, but its extraction remains a challenge, due to that kind of conservation damage the DNA quality. Extractions of these types of samples involve two different phases: Deparaffinization and DNA extraction. 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.

3. Kit Components

Item	AN0160	AN0160-XL
Deparaffinization solution	30 mL	150 mL
Tissue Lysis Buffer	10 mL	50 mL
Lysis / Binding Buffer	15 mL	75 mL
Proteinase K (*)	2 x 30 mg	10 x 30 mg
Desinhibition Buffer (**)	18 mL	90 mL
Wash Buffer (**)	10 mL	50 mL
Elution Buffer	10 mL	50 mL
MicroSpin Columns	50	250
Collection tubes	100	500

*Dissolve Proteinase K in water (1.5 ml) to obtain a 20 mg/mL stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed..

**Add the volume ethanol (96%-100%) specified [Not included] prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

4. Features

- 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, qPCR, NGS, STR analysis.

5. Storage specifications

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K at 4°C, all other kit components can be stored at room temperature.

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6. Product use Limitation

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to **www.canvaxbiotech.com** for Material Safety Data Sheet of the product.

DETAILED PROTOCOL

A) Deparaffinization Sample

1. Add **400 ul of Deparaffinization solution** to one FFPE tissue slice (10µm). ‼ *Make sure the sample does not comprise more than 15 mg paraffin.* **Vortex for 10 seconds.**
2. Incubate **3 minutes at 60° C** to promote the melting of the paraffin.
3. Vortex the sample immediately (at 60°C) 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 55C for 10 minutes** with caps open to evaporate the ethanol. Add 1.5 volumes of BL3 Buffer to the clarified lysate and mix vigorously by vortexing.

B) DNA Isolation

9. Add **180 µl of Tissue Lysis Buffer + 50µl Proteinase K**. Mix by vortexing 2-5 seconds. Incubate at 55°C for 1 hour or until the lysis is complete.
10. Incubate at 90°C for 1 hour. This incubation partially reverses formaldehyde modification of nucleic acids. If using only one heating block, leave the sample at room temperature after the 55°C incubation until the heating block has reached 90°C.
11. Briefly centrifuge to remove drops from the inside of the lid.
12. Add **200µl of Lysis/Binding Buffer**. Mix by vortexing. Incubate at 70°C for 5 minutes.
13. Add **200µl of Ethanol (96-100%)** to the lysate. Mix by vortexing. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
14. Transfer the lysate into reservoir of a combined MicroSpin Column –collection tube.
15. Centrifuge at 8000 rpm for 60 seconds. Remove the collection tube. If the sample is not drawn completely through the matrix, repeat the centrifugation step.
16. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition Buffer**.
17. Centrifuge at 12000-14000 rpm for 60 seconds. Remove the liquid.
18. Add **500 µl of Wash Buffer** into reservoir of MicroSpin column.
19. Centrifuge at 12000-14000 rpm for 60 seconds. Remove the liquid.
20. Centrifuge at maximum speed for 3 minutes to remove the residual ethanol.
21. Remove the collection tube and insert the MicroSpin column in a 1.5 ml microtube. Add **15- 30µl of Elution Buffer** (preheated at 70°C) directly onto the center of the silica membrane.
22. Incubate 1 minute. Centrifuge at maximum speed for 60 seconds. The microtube contains now genomic DNA.