PRODUCT INFORMATION

Thermo Scientific
GeneJET FFPE DNA Purification Kit
#K0881, #K0882

Lot __

Expiry Date __

Read Storage information (p. 4) before the first use!

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

The purity of isolated DNA from two sections (10 µm) of FFPE tissue is evaluated spectrophotometrically. The purified DNA has an A260/A280 ratio of 1.8–2.3. The functional quality of purified DNA is evaluated by PCR analysis.

Quality authorized by: Jurgita Zilinskiene

Rev.1 71

CONTENTS page

COMPONENTS OF THE KIT 4
STORAGE 4
DESCRIPTION 4
PRINCIPLE 4
IMPORTANT NOTES 5
ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED 5
PROTOCOL OF DNA PURIFICATION FROM FFPE SAMPLES 6
TROUBLESHOOTING 7
SAFETY INFORMATION 8

COMPONENTS OF THE KIT

GeneJET FFPE DNA Purification Kit	#K0881 50 preps	#K0882 250 preps
Proteinase K Solution	1.2 mL	4 × 1.3 mL
RNase A Solution	0.7 mL	3 × 1 mL
Digestion Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Binding Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Wash Buffer 1 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Wash Buffer 2 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Elution Buffer for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
GeneJET DNA Purification Columns & Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

The unopened vials of Proteinase K and RNase A solutions are stable at room temperature. Once the vial is opened, it should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

DESCRIPTION

Formalin fixed and paraffin embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples. GeneJET DNA Purification Kit for FFPE samples is designed for fast and convenient purification of DNA from various amounts of FFPE tissue. Up to 8 sections (10 µm thickness) of FFPE samples can be used for genomic DNA extraction without overnight incubation. Elimination of toxic reagents commonly used for deparaffinization allows an environmentally-friendly procedure. Each preparation recovers up to 8 µg of genomic DNA from one section that can be eluted in 20 µL to 80 µL of Elution Buffer. High quality eluted DNA can be directly used in downstream applications such as qPCR, PCR, NGS library preparation, or stored at -20 °C.

PRINCIPLE

Sections of FFPE samples are subjected to enzymatic digestion and lysis to liberate genomic DNA. The released DNA is decrosslinked by heat incubation. Subsequently, the resulting solution is centrifuged and the supernatant containing DNA is mixed with Binding Buffer. After addition of ethanol, the lysate is loaded onto the purification column. The adsorbed DNA is washed to remove contaminants and then eluted with the Elution Buffer.

IMPORTANT NOTES

- DNA yield and quality from FFPE tissue may vary considerably depending on the tissue source, the thickness of the slice, the age of the sample, post-sampling delay before fixation, fixation time, etc.
- Paraffin sections can be stored at or below 4 °C for 1 year without observable effects on DNA yield and usability. Longer-term storage of FFPE sections may have negative effect on the DNA due to oxidation.
- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples. For short–term, DNA solution may be stored at 0-4 °C, and for long-term at -20 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to the first use:

	#K0881 50 preps		**#K0882** 250 preps	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

After ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Check the **Digestion Buffer** and **Binding Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool it back down to 25 °C before use.
- Set two thermal heating-blocks or waterbaths, one at 65 °C and one at 90 °C.
- It is recommended to use microcentrifuge tubes with screw caps in the steps 1 to 4 (see the Genomic DNA purification from FFPE samples protocol, p.6).
- Wear gloves when handling the **Binding Buffer, Wash Buffer 1 and Proteinase K Solution** as these reagents contain irritants (*see* p.8 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex mixer
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes (for sample lysis and DNA elution)
- Centrifuge for 1.5 mL microcentrifuge tubes (≥ 12,000 × g)
- Thermal heating-blocks or waterbath (adjustable to 65 °C and 90 °C)
- Disposable gloves

PROTOCOL OF GENOMIC DNA PURIFICATION FROM FFPE SAMPLES

- This protocol describes how to extract DNA from one to eight sections of FFPE tissue (when each section is up to 10 µm thick).

Step	Procedure
1	Add 200 µL of Digestion Buffer to a microcentrifuge tube (not provided) containing one or more sections (up to eight) of FFPE tissue. Incubate for 3 min at 90 °C. During the incubation mix the sample a few times by gently shaking the tube. Make sure the tissue sections stay submerged in the solution. After incubation, mix thoroughly with a vortex mixer to completely dissolve the paraffin. Cool the sample down to room temperature. If necessary, spin down briefly to clear the lid. **Note. It is not necessary to cut off the excess paraffin. Use a microcentrifuge tube with a screw cap. Incubation time should be prolonged to 6 min if more than one section of FFPE tissue is used.**
2	Add 20 µL of Proteinase K solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down briefly to clear the lid.
3	Incubate the sample at 65 °C for 50 min in a thermoshaker or a water bath with occasional vortexing (300-400 rpm). **Note. Lysis time varies on the type and amount of FFPE sample processed. In some cases incubation time should be prolonged to 2 hours. Yield of DNA typically increases with extended lysis time**.
4	Transfer the samples to the heat block set to 90 °C and heat for 40 min. **Note. Prevent samples from being heated above 90 °C for a prolonged period of time.**
5	Centrifuge hot samples at 6000 × g for 1 min and transfer 200 µL of the digested lysate to a new 1.5 mL microcentrifuge tube (not provided). **Note. Transfer the entire liquid layer to a new tube leaving behind any wax particulates. Small amounts of debris will not affect the DNA yield. When using eight sections of FFPE tissue (each 10 µm thick), the digested lysate volume is 160-180 µL.**
6	Add 10 µL of RNase A solution and mix thoroughly by vortexing. Spin down briefly to clear the lid. Leave at room temperature for 10 min.
7	Add 200 µL of Binding Buffer. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
8	Add 400 µL of ethanol (96-100%) to the sample. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
9	Transfer the lysate to a GeneJET DNA Purification Column inserted into collection tube. Centrifuge for 1 min at 6000 × g. Discard the collection tube with the flow-through and place the column in a new collection tube (provided).
10	Add 500 µL of Wash Buffer 1 (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of Wash Buffer 2 (with ethanol added). Centrifuge for 3 min at maximum speed (≥ 12000 × g). Empty the collection tube, place the purification column back into the collection tube and re-spin the column for 1 min at maximum speed to dry the membrane. Discard the collection tube containing the flow-through solution and transfer the GeneJET DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not provided).

12	Add 60 µL of Elution Buffer directly to the center of the purification column membrane. Leave for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note. For maximum DNA yield, repeat the elution step with additional 60 µL of Elution Buffer. (perform the second elution using different tube).** **If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., one section of FFPE sample) the volume of the Elution Buffer added to the column can be reduced to 20 µL. Elution volumes in the range of 20-80 µL are recommended, the default volume is 60 µL.**
13	Discard the column. Use the purified DNA immediately in downstream applications or store at -20 °C.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue than indicated in lysis protocols. **Starting material was not completely digested.** If the suspension does not clarify during Proteinase K digestion, this could indicate that it is oxidized. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain. **Ethanol was not added to the lysate.** Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5. **Poor sample quality.** Sample fixation, embedding and storage have a significant impact on quality and amount of the DNA in FFPE tissue samples.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	**Excess sample was used during lysate preparation.** Too much starting material was used. Overloading may lead to a decrease in DNA yield. **Tissue was not completely digested.** Insufficient disruption and / or homogenization of starting material. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** Do not let the flow-through touch the column outlet after the second wash with Wash Buffer 2. Always re-spin the column for an additional 1 min. at maximum speed (≥ 12000 × g) after the second wash. **Purified DNA contains residual salt.** Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer 1 first and then proceed to wash with Wash Buffer 2.

SAFETY INFORMATION

Proteinase K

Danger
Hazard statements:
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements:
P285 In case of inadequate ventilation wear respiratory protection.
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Binding Buffer

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Wash Buffer 1

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.
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