GeneJET™ FFPE DNA Purification Kit

Catalog Numbers K0881, K0882

Pub. No. MAN0012673 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ GeneJET™ FFPE DNA Purification Kit 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.

Technology overview

Sections of FFPE samples are subjected to enzymatic digestion and lysis to liberate genomic DNA. The released DNA is de-crosslinked 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 bound DNA is washed to remove contaminants and then eluted with the Elution Buffer.

Contents and storage

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

Table 1 GeneJET™ FFPE DNA Purification Kit

Item	Cat. no. K0881 (50 reactions)	Cat. no. K0882 (250 reactions)	Storage
Proteinase K Solution	1.2 mL	4 × 1.3 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
RNase A Solution	0.7 mL	3 × 1 mL	
Digestion Buffer	11 mL	55 mL	15–25°C
Binding Buffer	11 mL	55 mL	
Wash Buffer 1 (concentrated)	10 mL	40 mL	
Wash Buffer 2 (concentrated)	10 mL	40 mL	
Elution Buffer	10 mL	40 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns and Collection Tubes	50	250	Store at 15–25°C for up to 6 months. For longer periods, store at 2–8°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex mixer, or equivalent	MLS
Thermo heating-blocks or waterbath (adjustable to 65°C and 90°C)	MLS
Centrifuge capable of ≥12,000 × *g* for 1.5 mL microcentrifuge tubes	MLS
Tubes and other consumables	
Microcentrifuge tubes (1.5 mL) with screw caps	MLS
Disposable gloves	MLS
Ethanol, 96–100% (molecular biology grade)	MLS

Procedural guidelines

IMPORTANT! Wear gloves when handling the Lysis Solution, Wash Buffer I, and Proteinase K Solution as these reagents contain irritants.

- 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.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

–	Cat. no. K0881 (50 reactions)		Cat. no. K0882 (250 reactions)	
–	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
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

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

Before each use

- 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 temperature for two thermal heating-blocks or waterbaths, one at 65°C and one at 90°C.

Purify genomic DNA from FFPE samples

This protocol describes how to extract DNA from one to eight sections of FFPE tissue for sections up to 10 µm thick.

1. Add 200 µL of Digestion Buffer to a microcentrifuge tube (with screw cap to prevent evaporation) containing one or more sections (up to eight) of FFPE tissue.
2. Incubate for 3 minutes at 90°C. During the incubation, mix the sample a few times by gently shaking the tube.

   Note: Ensure tissue sections stay submerged in the solution.

3. 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.

4. Add 20 µL of Proteinase K solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down briefly to clear the lid.

5. Incubate the sample at 65°C °C for 50 minutes in a thermoshaker or a water bath with occasional vortexing.

   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.

6. Transfer the samples to the heat block set to 90°C then incubate for 40 minutes.

   Note: Prevent samples from being heated above 90°C for a prolonged period of time.

7. Centrifuge incubated samples at 6000 × *g* for 1 minute then transfer 200 µL of the digested lysate to a new 1.5 mL microcentrifuge tube.

   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.

8. Add 10 µL of RNase A solution then mix thoroughly by vortexing. Spin down briefly to clear the lid then incubate at room temperature for 10 minutes.

9. Add 200 µL of Binding Buffer then vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.

10. Add 400 µL of ethanol (96-100%) to the sample then vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.

11. Transfer the lysate to a GeneJET™ DNA Purification Column inserted into collection tube.

12. Centrifuge column at 6000 × *g* for 1 minute. Discard the collection tube with the flow through then place the column in a new collection tube.

13. Add 500 µL of Wash Buffer 1 with ethanol added then centrifuge at 8000 × *g* for 1 minute. Discard the flow-through then place the purification column back into the collection tube.

14. Add 500 µL of Wash Buffer 2 with ethanol added then centrifuge at ≥ 12000 × *g* for 3 minutes.

15. Empty the collection tube then place the purification column back into the collection tube. Re-spin the column for 1 minute at maximum speed to dry the membrane.

16. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

17. Add 60 µL of Elution Buffer directly to the center of the purification column membrane. Leave for 2 minutes at room temperature then centrifuge at 8000 × *g*vfor 1 minute.

    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.

18. Discard the column. Use the purified DNA immediately in downstream applications or store at -20 °C.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified DNA	Excess sample was used during lysate preparation.	Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols.

Observation	Possible cause	Recommended action
Low yield of purified DNA *(continued)*	The 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 visible in solution.
	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 added to 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. See “Before first use of the kit” on page 2.
	Poor sample quality.	Sample fixation, embedding and storage have a significant impact on quality and amount of the DNA in FFPE tissue samples.
	Columns stored at room temperature (15–25°C) for longer than 6 months.	Extended room temperature storage (15–25°C) for columns beyond 6 months may lead to membrane drying and reduced DNA yield. For prolonged storage exceeding 6 months, it is recommended to store columns at 2–8°C. See “Contents and storage” on page 1.
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 minute 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.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0012673 B

Revision	Date	Description
B	10 May 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The storage conditions for columns and collection tubes were updated. • The version format was changed in conformance with internal document control procedures.
1.0	28 February 2014	New document for the GeneJET™ FFPE DNA Purification Kit.