GeneJET™ Whole Blood Genomic DNA Purification Mini Kit

Catalog Numbers K0781, K0782

Pub. No. MAN0012667 Rev. D

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™ Whole Blood Genomic DNA Purification Mini Kit is designed for rapid and efficient purification of high-quality genomic DNA from whole blood and related body fluids. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA greater than 30 kb in size. Isolated DNA can be used directly in PCR, qPCR, Southern blotting and enzymatic reactions.

Samples are digested with Proteinase K in the supplied Lysis Solution. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared Wash Buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1 Typical genomic DNA yields from various sources

Source	Amount	DNA yield (µg)
Human blood	200 µL	2–10
Avian blood (chicken)	5 µL	20
Mouse blood	200 µL	2–4
Rat blood	200 µL	2
Rabbit blood	200 µL	4–7
Bone marrow	200 µL	10–65
Buffy coat	200 µL	4–13
Dried blood	200 µL	0.05–0.28
Buccal Swabs	100 µL	0.05–0.12

Contents and storage

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

Table 2 GeneJET™ Whole Blood Genomic DNA Purification Mini Kit

Item	Cat. no. K0781 (50 reactions)	Cat. no. K0782 (250 reactions)	Storage
Proteinase K Solution	1.2 mL	5 × 1.2 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
Lysis Solution	24 mL	120 mL	15–25°C
Wash Buffer I (concentrated)	10 mL	40 mL	
Wash Buffer II (concentrated)	10 mL	40 mL	
Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns preassembled with 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: Fisher Scientific (**fisherscientific.com**) or another major laboratory supplier.

Table 3 For biofluid or transport media sample protocols

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex mixer, or equivalent	MLS
Thermomixer with associated block (1.5 mL)	MLS
Centrifuge capable of ≥16,000 × *g* for microcentrifuge tubes	MLS
Tubes and other consumables	
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes	13-698-791
Corning™ Costar™ Snap Cap Microcentrifuge Tubes	07-200-210
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips
Disposable gloves	MLS
Ethanol, 96–100% (molecular biology grade)	MLS
PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)	MLS
TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	MLS

Procedural guidelines

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

- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20°C or -70°C.
- Check the Lysis Solution for salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, then cool back down to 25°C before use.

- Typically, the purified genomic DNA has an $A_{260/280}$ ratio between 1.7 and 1.9, however, when DNA concentration is lower than 20 ng/µL, deviations from the expected ratio are occasionally observed.
- Adjust the sample volume to 200 µL with 1X PBS or TE buffer.
- Centrifugation speed in rpm is given for 24-place microcentrifuges.

Before first use of the kit

Add the indicated volume of ethanol (96-100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) before first use.

–	Cat. no. K0781 (50 reactions)		Cat. no. K0782 (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

Purify whole blood genomic DNA

1. Add 20 µL of Proteinase K Solution to 200 µL of whole blood, then mix by vortexing.
2. Add 400 µL of Lysis Solution, then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

   Note: If using less than 200 µL of blood, adjust sample volume to 200 µL with 1X PBS or TE buffer (not provided). If using larger volumes, see “Purify large volumes of whole blood genomic DNA” on page 3.
3. Incubate the sample at 56°C for 10 minutes while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed.
4. Add 200 µL of ethanol (96–100%), then mix by pipetting.
5. Transfer the prepared mixture to the spin column, then centrifuge at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution, then place the column into a new 2 mL collection tube (included).

   IMPORTANT! Do not exceed specified relative centrifugal force.

   Note: Close the bag with GeneJET™ Genomic DNA Purification Columns tightly after each use.
6. Add 500 µL of Wash Buffer WB I (with ethanol added), then centrifuge 8,000 × *g* for 1 minute. Discard the flow-through and place the column back into the collection tube.
7. Add 500 µL of Wash Buffer II (with ethanol added) to the column, then centrifuge at ≥20,000 × *g* for 3 minutes.
8. Empty the collection tube, then place the purification column back into the tube. Centrifuge at ≥20,000 × *g* for 1 minute.
9. Discard the collection tube containing the flow-through solution, then transfer the column to a sterile 1.5 mL microcentrifuge tube (not included).
10. Add 200 µL of Elution Buffer to the center of the column membrane to elute genomic DNA. Incubate for 2 minutes at room temperature, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µl of Elution Buffer.
    - If more concentrated DNA is required or if DNA has been isolated from a small amount of starting material (e.g., 50 µL) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that lower volumes of Elution Buffer will result in lower final yield of eluted DNA.
11. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20°C.

Purify large volumes of whole blood genomic DNA

For purification of DNA from samples exceeding the standard 200 µL volume, it is necessary to burst red blood cells prior to performing the cell lysis step. Up to 500 µL of mammalian blood can be processed using following protocol:

1. Add 1 mL of ice-cold nuclease-free water to 500 µL of whole blood, then mix thoroughly by vortexing or pipetting.
2. Incubate the sample for 5 minutes at room temperature.
3. Centrifuge at 800 × *g* for 5 minutes, then discard the supernatant.
4. Resuspend the pellet in 200 µL of 1X PBS.
5. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3.

Purify genomic DNA from nucleated blood

Nucleated avian or fish blood contains very large amounts of genomic DNA and therefore the volume of the staring material has to be scaled down. The DNA purification procedure follows the same protocol as mammalian blood, except that 2–10 µL of blood are used per purification.

1. Using 2-10 µL of nucleated blood, adjust the volume to 200 µL with 1X PBS.
2. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3.

Purify genomic DNA from buccal swabs

1. To collect a sample, scrape the swab 5-6 times against the inside cheek.
2. Swirl the swab for 30–60 seconds in 200 µL of 1X PBS.
3. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3,

Purify genomic DNA from bone marrow

1. Harvest 25–200 µL of fresh or frozen bone marrow.
2. Adjust the volume to 200 µL with 1X PBS.
3. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3.

Purify genomic DNA from dried blood spots

1. Cut out the section of filter containing the dried blood sample and place into a microcentrifuge tube.
2. Add 200 µL of 1X PBS and incubate 5–10 minutes at room temperature.
3. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3.

Purify genomic DNA from buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood and contains approximately 5–10 times more DNA than an equivalent volume of whole blood. After centrifugation, 3 different fractions are distinguishable: the upper clear layer containing plasma; the intermediate buffy coat layer containing concentrated leukocytes, and the bottom layer containing concentrated erythrocytes.

1. Centrifuge 1.5 mL of whole blood at 2,500 × *g* for 10 minutes at room temperature. Three layers should be visible.
2. Remove upper clear layer by aspiration.
3. Collect approximately 200 µL of intermediate layer using an automatic pipette.

   Note: If necessary, adjust the volume to 200 µL with 1X PBS.
4. Proceed to step 1 of "Purify whole blood genomic DNA" on page 3.

Purify genomic DNA from urine

1. Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).
2. Centrifuge at 800 × *g* for 10 minutes, then discard the supernatant.
3. Resuspend the pellet in 200 µL of 1X PBS.
4. Proceed to step 1 of “Purify whole blood genomic DNA” on page 3.

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.
	The starting material was not completely digested.	Extend the Proteinase K digestion at 56°C until complete lysis occurs and no particles remain visible in solution.
	Sample was not thoroughly mixed with lysis buffer and Proteinase K.	The mixture has to be vortexed or pipetted immediately after adding lysis buffer.
	Ethanol was not added to the lysate.	Ensure that ethanol is 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.	Ensure that ethanol is added to Wash Buffer WB I and Wash Buffer II before use. See “Before first use of the kit” on page 3.
	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 2.
Purified DNA is degraded	Sample was frozen and thawed repeatedly.	Avoid repeated sample freeze / thaw cycles. Use a fresh sample for DNA isolation. Perform extractions from fresh material when possible..
	Inappropriate sample storage conditions.	Whole blood can be stored at 4°C for no longer than 1–2 days. For long term storage, blood samples should be aliquoted in 200 µL aliquots and stored at -20°C.
RNA contamination	The sample was RNA-rich.	With the GeneJET™ Whole Blood Genomic DNA Purification Mini Kit , the optimized buffers in combination with silica membrane technology allows for purification of essentially RNA-free gDNA without RNase treatment. However, when working with extremely transcriptionally active cell types, e.g. bone marrow, some RNA contamination might occur. If absolutely RNA-free DNA is necessary, add 20 µL of RNase A solution (10 mg/mL) to the sample prior to the addition of lysis buffer. See “Purify whole blood genomic DNA” on page 3.
Inhibition of downstream enzymatic reactions	Purified DNA contained residual ethanol.	If residual solution is observed in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column at ≥20,000 × *g* for 1 minute.
	Purified DNA contained residual salt.	Use the correct order for the Wash Buffers. Always wash the purification column with Wash Buffer WB I first and then proceed with Wash Buffer II.

Limited product warranty

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Revision history: Pub. No. MAN0012667 D

Revision	Date	Description
D	10 May 2024	• The storage conditions for columns and collection tubes were updated. • The version format was changed in conformance with internal document control procedures.
C.0	17 October 2022	The user manual was updated to current style guidelines. The required materials not supplied table was updated to include recommendations for microcentrifuge tubes.