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PRODUCT INFORMATION

Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit #K0781, #K0782

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⚠ Read Storage information (p. 2) before first use!

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For Research Use Only. Not for use in diagnostic procedures.

#_
Lot _
Exp. _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit is qualified by isolating genomic DNA from 200 µL of blood following the protocols outlined in the manual. The purified genomic DNA has an $A_{260/280}$ ratio between 1.7 and 1.9. A single band of more than 30 kb is observed after agarose gel electrophoresis and ethidium bromide staining. The functional quality of purified genomic DNA is evaluated by PCR amplification of a single-copy gene and by digestion with restriction enzymes.

Quality authorized by: Jurgita Žilinskienė

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COMPONENTS OF THE KIT

GeneJET Whole Blood Genomic DNA Purification Mini Kit	#K0781 50 preps	#K0782 250 preps
Proteinase K Solution	1.2 mL	5 × 1.2 mL
Lysis Solution	24 mL	120 mL
Wash Buffer WB 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
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes (2 mL)	50	250

STORAGE

Proteinase K solution is stable at room temperature as long as the vial remains sealed. After the vial is opened, proteinase K should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!

DESCRIPTION

The 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. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

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	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	100 µL	0.05-0.28
Buccal Swabs	-	0.05-0.12

IMPORTANT NOTES

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

	#K0781 50 preps		**#K0782** 250 preps	
	Wash Buffer WB I	**Wash Buffer II**	**Wash Buffer WB 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

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

- 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.
- Wear gloves when handling the **Lysis Solution and Wash Buffer I** as these reagents contain irritants.
- 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 (not provided).
- Centrifugation speed in rpm's is given for 24-place microcentrifuges.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Thermomixer, shaking water bath or rocking platform capable of heating up to 56 °C
- Disposable gloves.

Buffers

For sample volume adjustment:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

PROTOCOLS

Protocols for genomic DNA purification from buccal swabs, buffy coat, dried blood spots, body fluids, and avian blood are described on p.5-7.

A. Whole Blood Genomic DNA Purification Main Protocol

Step	Procedure
1	Add 20 µL of Proteinase K Solution to 200 µL of whole blood, mix by vortexing. Add 400 µL of Lysis Solution, 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, follow the protocol on page 5.
2	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.
3	Add 200 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer the prepared mixture to the spin column. Centrifuge for 1 min at 6,000 × g (~8,000 rpm). Discard the collection tube containing the flow-through solution. 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!
5	Add 500 µL of Wash Buffer WB I (with ethanol added). Centrifuge for 1 min at 8,000 × g (~10,000 rpm). Discard the flow-through and place the column back into the collection tube.
6	Add 500 µL of Wash Buffer II (with ethanol added) to the column. Centrifuge for 3 min at maximum speed (≥20,000 × g, ≥14,000 rpm). *<u>Recommended:</u> Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm).* Discard the collection tube containing the flow-through solution and transfer the column to a sterile 1.5 mL microcentrifuge tube (not included).
7	Add 200 µL of Elution Buffer to the center of the column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8,000 × g (~10,000 rpm). **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.
8	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

B. DNA Purification from Large Volumes of Whole Blood

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:

Step	Procedure
1	Add 1 mL of ice cold nuclease free water to 500 µL of whole blood, mix thoroughly by vortexing or pipetting.
2	Incubate the sample for 5 min at room temperature.
3	Centrifuge for 5 min at 800 × g (~3,000 rpm).
4	Discard the supernatant.
5	Resuspend the pellet in 200 µL of 1 x PBS.
6	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

C. DNA Purification 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.

Step	Procedure
1	Take 2-10 µL of nucleated blood.
2	Adjust the volume to 200 µL with 1 × PBS.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

D. DNA Purification from Buccal Swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 1 × PBS.
3	Go to step 1 of the standard Whole Blood Genomic DNA Purification Protocol (p.4).

E. DNA Purification from Bone Marrow

Step	Procedure
1	Harvest 25-200 µL of fresh or frozen bone marrow.
2	Adjust the volume to 200 µL with 1 × PBS.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

F. DNA Purification from Dried Blood Spots

Step	Procedure
1	Cut out the section of filter containing the dried blood sample and place into a microcentrifuge tube.
2	Add 200 µL of 1 × PBS and incubate 5-10 min at room temperature.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

G. DNA Purification 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. Prepare the buffy coat by centrifuging whole blood at 2,500 × g for 10 min at room temperature. 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.

Step	Procedure
1	Centrifuge 1.5 mL of whole blood at 2,500 × g (~5,000 rpm) 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 1× PBS.
4	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

H. DNA Purification from Urine

Step	Procedure
1	Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).
2	Centrifuge 10 min at 800 × g (~3,000 rpm).
3	Discard the supernatant.
4	Resuspend the pellet in 200 µL of 1 × PBS.
5	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

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 blood than indicated in lysis protocols. **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 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.** Ensure that ethanol was added to Wash Buffer WB I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on p.3.
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	**RNA-rich sample** With the GeneJET Genomic DNA Purification Mini Kit, the optimised 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 (step 1, p. 4).
Inhibition of downstream enzymatic reactions	**Purified DNA contains 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 for an additional 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm). **Purified DNA contains 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.

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