DNAbioTech
Life Science

DNAbiotech
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Blood Genomic DNA

Catalog no.: DB9817

(50 and 100 prep)

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Customer and technical support

If you have any questions, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

About this user manual

It is recommended for first-time users to read the detailed protocol sections of the user manual before using these products. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

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General description

DNAbiotech's "**Blood Genomic DNA extraction kit"** is developed for DNA extraction from whole blood, cultured cells, serum, plasma, or other body fluids. Lysis is achieved by incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **DNAbiotech Blood Columns** are achieved by the addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations with the "**Blood Genomic DNA extraction kit"** washing buffers.

Kit specifications

-The blood Genomic DNA extraction kit is designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids.

- DNA can be purified successfully from blood samples treated with EDTA, citrate, or heparin. If leukocyte-rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile **PBS** (DB0010).

-The kits allow purification of highly pure genomic DNA with a typical concentration of 30–50 ng per µL.

-The obtained DNA is ready to use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reaction.

Warranty: The 1st reaction of this product may be used as a free sample and if desired results do not obtain the product could be returned**.**

Quality Control

In accordance with DNAbiotech Co. Management System, each part of the **Blood Genomic DNA extraction kit** is tested against predetermined specifications to ensure consistent product quality**.**

Safety Notes

The buffers included in **the Blood Genomic DNA extraction kit** contain an irritant that is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protectors, and follow standard safety precautions.

Buffer L1 contains chaotropic agents. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste**.**

Storage of blood samples

For the extraction of genomic DNA from blood treated with anticoagulants (heparin, citrate, or EDTA) using a DNAbiotech Genomic Blood kit the blood samples can be stored at room temperature, +4 °C, or frozen. Blood samples stored at room temperature or +4 °C for up to several days or weeks, respectively, will still allow DNA isolation. However, DNA yield and quality will slowly decrease due to the prolonged storage of blood samples under these conditions. Blood stored frozen for years is well suited for DNA isolation. However, the highest yields and quality of DNA are obtained from fresh blood.

Elution procedures

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal to or higher than 8. This will increase DNA stability, especially during the long-term and/or multi-use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. For optimal performance of isolated DNA in subsequent downstream

applications, we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Convenient elution: For convenience, an elution buffer of ambient temperature may be used. This will result in a lower yield (approximately 20 %) compared to elution with preheated elution buffer.

High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid can be eluted.

High concentration: Perform one elution step with 60 % of the volume indicated in the individual protocol. The concentration of DNA will be higher than with standard elution.

High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol; incubate for 3 min at 60° C and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–90 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.

Kit Components

No.	Name	cat #: DB9822-50rxn	cat #: DB9822-100 rxn
1	*Handbook protocol*	1	1
2	Columns and Collection Tubes (pcs)	50	100
3	Collection Tubes (pcs)	100	200
4	L1 Buffer (Lysis Buffer)	11 ml	22 mL
5	Proteinase K* (Lyophilized)	As needed	As needed
6	Proteinase K buffer*	As needed	As needed
7	VB2 Solution	17 ml	33 ml
8	WB1	3 7 ml	75 ml
9	WB2	3 7 ml	75 ml
10	Elution buffer (EB)	5 ml	10 ml

* Proteinase K buffer should be mixed with lyophilized proteinase K and then stored at –20° C for up to 12 months.

Note: During storage, especially at low temperatures, a precipitate may form in some Buffers. Such precipitates can be easily dissolved by incubating the bottle at 60 °C.

Storage condition:

Shipping: RT

Storage: The reconstituted proteinase K should be stored at – 20° C. All other kit components can be stored at room temperature (18–25 °C) and are stable for up to one year.

Protocols of Genomic DNA purification

Before experiment notes:

* Check if Washing Buffers and Proteinase K be prepared according to the procedure.

*Set an incubator, thermos-block, or water bath to 56 °C.

*Preheat Elution Buffer BE to 56 °C.

* Centrifuge speed: 8000- 12000 *x g*

A. Lysis blood sample

1. Pipet 200 µl anticoagulated blood into a 1.5 ml or 2 ml microcentrifuge tube. For sample volumes less than 200 ul, add ***PBS*** to adjust the volume to 200 ul.
2. Add 20 µl proteinase K to the sample.
3. Add 200 µl L1 Buffer **Mix thoroughly** by vortexing, and incubate at 56°C for 20 min. **Vigorous vortexing** should be repeated **every 4 minutes**. Spin down briefly to remove any drops from the inside of the lid.

Note: The lysate should become brownish during incubation with Lysis Buffer. Vigorous mixing is important to obtain a high yield and purity of DNA.

Increase incubation time with proteinase K (up to 30 min) and vortex several times vigorously during incubation if processing older or clotted blood samples.

B. Adjust DNA binding conditions

4. Add 300 µl of absolute ethanol to the sample, and mix thoroughly by inverting. Incubate for 1 min at room temperature. Spin down briefly to remove any drops from the inside of the lid.

C. DNA binding

5. Transfer the mixture from step 4 into the column placed in a 2 ml collection tube. Centrifuge for 1 min at 8000 *x g*. Discard flow-through.

D. Wash silica membrane

6. Place the column into a new collection tube, add 700 µl Wash Buffer1, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.
   Note: at this step, if the color of the filter membrane of the spin column remained brownish, repeat the step.
7. Place the column in a new 2 ml collection tube, add 700 µl Wash Buffer 2, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.

E. Dry silica membrane

8. Place the column into the collection tube, then centrifuge at full speed (>12,000 *x g*) for 3 min to dry the membrane completely. Discard flow-through and collection tube. Residual ethanol is removed during this step.
   Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

F. Elute highly pure DNA

9. Place the column in a clean 1.5 ml microcentrifuge tube. Carefully open the lid of the column and apply **40-50 ul** prewarmed Elution Buffer to the center of the membrane. Incubate at RT for 3 min.
   Note: for better elution close the lid and incubate at 56 °C for 1-2 min. Centrifuge at full speed (>12,000 *x g*) for 1 min.
10. Apply 3-5 ul of eluted DNA into a 1% agarose gel and check the quality. You can use DNAbiotech Safe stain (DB9738) to color the DNA.

Procedure at a glance:

Steps	Genomic Blood DNA Extraction at a glance	
1 **Lyse blood samples**		**200 µL blood** **20 µL Pro. K** **200 µL L1** **Mix** **About 20 min at 56 °C**
2 **Adjust DNA binding conditions**		**300 µL ethanol**
3 **Bind DNA**		**Load all** **1 min** **8,000 x *g***
4 **Wash silica membrane** **1st: 600 ul WB1** **2nd: 600 ul WB2**		**Load all** **1 min** **8,000 x *g***
5 **Dry silica membrane**		**3 min** **12000 x *g***
6 **Elute highly pure DNA**	 	**50 ul EB** **56° C 3 min** **12000 x *g*** **1min**
7 QC	**Load 5 ul eluted DNA on 1% agarose gel** Stain DNA with DNAbiotech ECO DNA safe stain	

Troubleshooting

Problem	Possible cause	suggestions
No or Low DNA yield Or the poor quality of DNA	*Low concentration of leukocytes in sample*	Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (4000 x *g*; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat).
	Incomplete cell lysis	a. Sample not thoroughly mixed with lysis reagent or Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of lysis buffer. b. Proteinase K digestion is not optimal. Never add Proteinase K directly to the lysis buffer. Incubate for 15–20 min at 60 °C- 65 °C.
	Reagents not applied properly	Prepare buffers and Proteinase K solution according to instructions. Be sure to add ethanol to lysates before loading them on columns.
	Suboptimal elution of DNA from the column	a. Preheat Buffer BE to 60-70 °C before elution. Add EB directly onto the center of the silica membrane. b. Elution efficiencies decrease dramatically if elution is performed with buffers of pH < 7.0. Use a slightly alkaline elution buffer like EB (pH 8.5). c. Mix vigorously during the 60 °C / 70 °C incubation step, especially when working with older or clotted blood samples.
	RNA in sample	If RNA-free DNA is desired, add 20 µL RNase A solution (10 mg/mL) (cat #: DB9700) before the addition of lysis buffer.
	Old or clotted blood samples processed	To extract DNA from older or clotted blood samples, we recommend prolonging Proteinase K incubation to 30 min and vortexing several times during this step. performance can be improved by the following steps: First, incubate the lysate for 10–15 min at room temperature. Incubate for 15 min at the recommended 60 °C afterward. Clear lysate before the addition of ethanol. It is recommended to perform a short centrifugation step of about 30–60 s after the lysis of the sample material (before the addition of

		ethanol) to pellet non-lysed clumps. In the case of difficult blood samples, it might happen that the washing steps with ethanolic WB2 are not sufficient to remove all contamination. An additional wash step with a buffer including chaotropic salt is recommended, for example, water / WB 1 / ethanol mix (1:1:1). Afterwards, the washing step with ethanolic Buffer WB 2 and 3 should be performed to completely remove the chaotropic salt of the wash buffer.
Suboptimal Performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol* *Contamination of DNA with inhibitory substances*	Be sure to remove all of the ethanolic Buffer before eluting the DNA. If the level of WB after the second wash has reached the column outlet for any reason, discard the flow-through, place the column back into the Collection Tube, and centrifuge again. If DNA has been eluted with Tris / EDTA-buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in Buffer BE. If the A260 / A280 ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer WB 3 plus 1 volume ethanol to the eluate, load on a spin column, and centrifuge for 1 min at 10000 x g.

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