

Whole blood Genomic DNA Extraction/Isolation Kit

Cat. No. OttoP9510

For technical support please contact us info@ottosci.com

Summary & Explanation

The Whole Blood Genomic DNA Extraction Kit is designed for the rapid and efficient purification of high-quality genomic DNA from up to 200 µL of whole blood, peripheral blood mononuclear cells (PBMCs).

Genomic DNA purification is based on silica membrane spin-column technology, eliminating the need for hazardous phenol/chloroform extraction, time-consuming alcohol precipitation, or expensive resin-based purification methods.

Spin columns selectively bind DNA under optimized high-salt conditions. Following the removal of contaminants through washing steps, the bound DNA is efficiently eluted under low-salt, mildly alkaline conditions.

Following cell lysis, the Main Protocol can be completed in less than 30 minutes and yields purified genomic DNA with fragment sizes greater than 30 kb.

The purified genomic DNA is suitable for direct use in a wide range of downstream applications, including PCR, qPCR, Southern blotting, and enzymatic reactions.

Storage

- All solutions included in the kit can be stored at room temperature (15–25°C) for up to 12 months, provided that the bottles are tightly closed to prevent exposure to air.
- To maintain the stability and activity of Proteinase K throughout the specified shelf life, store the Proteinase K Solution at 2–8°C after opening.

IMPORTANT NOTES

- To minimize DNA degradation, avoid repeated freeze–thaw cycles of the samples. For optimal extraction efficiency, use fresh material or samples that have been immediately frozen and stored at –20°C or –80°C.
- Wash Solution 1 and Wash Solution 2 are supplied as concentrated buffers and must be diluted with absolute ethanol (≥96%) before use.

TROUBLESHOOTING

Problem	Possible Causes and Solutions
DNA Degradation	Sample was subjected to repeated freeze–thaw cycles. Avoid repeated freeze–thaw cycles. Use a fresh sample for DNA isolation. Whenever possible, perform the extraction using fresh material. Improper storage conditions. Whole blood may be stored at 4°C for no longer than 1–2 days. For long-term storage, aliquot blood samples into 200 µL portions and store at –20°C.
RNA Contamination	High levels of residual RNA are present. If RNA-free DNA is required for the intended application, add 20 µL of RNase A solution (10 mg/mL) to the sample before adding the lysis buffer.
Inhibition of Enzymatic Reactions	Purified DNA contains residual ethanol. If residual alcohol remains in the column after the final wash step, transfer the column to a new collection tube and centrifuge at maximum speed ($\geq 20,000 \times g$; $\geq 14,000$ rpm) for 1 minute. Purified DNA contains residual salts. Ensure that all wash steps are performed using the specified number of washes and the indicated volumes.
Low DNA Yield	Excessive sample volume was used during the lysate preparation step. Reduce the amount of sample used. Do not exceed the sample volumes specified in the protocol. Undigested starting material is present. Ensure that Proteinase K digestion is performed at 56°C. If necessary, extend the incubation time until complete digestion is achieved. Insufficient mixing of the sample in the lysate. If the contents are not fully mixed after vortexing, homogenize the lysate by pipetting. Ethanol was not added. Ensure that ethanol is added to the lysate and mixed thoroughly. Also ensure that ethanol has been added to the wash buffers before use.

WASH BUFFER PREPARATION

Ethyl Alcohol (absolute %99.9) Addition Guide

Wash Buffer-1 (WB-1)

Kit Size	Wash Buffer 1 (WB-1)	Absolute Ethanol	Final Volume
50 Tests	18 mL	11 ml	29 ml
100 Tests	36 mL	22 ml	58 ml
250 Tests	80 ml	49 ml	129 ml

Wash Buffer-2 (WB-2)

Kit Size	Wash Buffer 2 (WB-2)	Absolute Ethanol	Final Volume
50 Tests	17 mL	41 ml	58 ml
100 Tests	34 mL	82 ml	116 ml
250 Tests	77 ml	180 ml	257 ml

Note: Add the indicated volume of absolute ethanol to the wash solution concentrate before first use.

KIT COMPONENTS

Whole Blood Genomic DNA Cat. No.: OttoP9510	250 Tests OttoP9510-250	100 Tests OttoP9510-100	50 Tests OttoP9510-50
Lysis Buffer (LB)	65 mL	26 mL	13 mL
Proteinase K Buffer	5 × 1.30 mL	2 × 1.30 mL	1.30 mL
Wash Buffer-1 (WB-1)	80 mL	36 mL	18 mL
Wash Buffer-2 (WB-2)	77 mL	34 mL	17 mL
Spin Columns	250	100	50
Elution Buffer (EB)	30 mL	14 mL	10 mL

WHOLE BLOOD GENOMIC DNA EXTRACTION

MAIN PROTOCOL



i All centrifugation steps: **8,000 $\times$ g** (approx. 1 minute = ~10,000 rpm, depending on rotor).

Description of Protocol

1. Sample Preparation

In a 1.5 mL microcentrifuge tube, add the following components in the specified order:

- a. PK Solution: 25 μ L
- b. Whole Blood: 200 μ L
- c. LB Solution: 250 μ L

Note: Add components a–b–c sequentially in the order indicated. The use of whole blood collected in an EDTA tube is recommended.

2. Lysis

Vortex for 15 seconds until all components are thoroughly mixed and the lysate is homogeneous.

3. Incubation

Incubate at 56°C for 5 minutes.

4. Ethanol Addition

Add 250 μ L absolute ethanol.

Mix the lysate thoroughly by pipetting until homogeneous.

5. Binding

Transfer the entire lysate to the spin column.

Centrifuge at 8,000 $\times$ g for 1 minute.

Discard the flow-through and replace the collection tube.

6. Wash 1

Add 500 μ L Wash Buffer-1 (WB-1) to the spin column.

Centrifuge at 8,000 $\times$ g for 1 minute.

Discard the flow-through and replace the collection tube.

7. Wash 2

Add 500 μ L Wash Buffer-2 (WB-2) to the spin column.

Centrifuge at 8,000 $\times$ g for 1 minute.

Discard the flow-through and replace the collection tube.

8. Second Wash / Dry Spin

Add 500 μ L WB-2 Solution to the spin column.

Centrifuge at 8,000 $\times$ g for 2 minutes.

Transfer the spin column to a clean microcentrifuge tube for DNA elution.

9. Elution

Add 50–100 μ L EB Solution directly to the center of the spin-column membrane.

Incubate at room temperature for 1 minute.

10. Final Centrifugation

Centrifuge at 8,000 $\times$ g for 1 minute.

The eluate contains the purified genomic DNA and is ready for downstream applications.