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), and dried blood spots.

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 (≥20,000 × g; ≥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.

Description of Protocol

1. Sample Preparation
In a 1.5 mL microcentrifuge tube, add the following components in the specified order:

a. PKD Solution: 25 µL
b. Whole Blood: 200 µL
c. LBD Solution: 250 µL

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 µL absolute ethanol (>96%).

Mix the lysate thoroughly by pipetting until homogeneous.

5. Binding
Transfer the entire lysate to the spin column.

Centrifuge at 8,000 × g for 1 minute.

Discard the flow-through and replace the collection tube.

6. Wash 1
Add 500 µL WBD-1 Solution to the spin column.

Centrifuge at 8,000 × g for 1 minute.

Discard the flow-through and replace the collection tube.

7. Wash 2
Add 500 µL WBD-2 Solution to the spin column.

Centrifuge at 8,000 × g for 1 minute.

Discard the flow-through and replace the collection tube.

8. Second Wash / Dry Spin
Add 500 µL WBD-2 Solution to the spin column.

Centrifuge at 8,000 × g for 2 minutes.

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

9. Elution
Add 50–100 µL EBD Solution directly to the center of the spin-column membrane.

Incubate at room temperature for 1 minute.

10. Final Centrifugation
Centrifuge at 8,000 × g for 1 minute.

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

WHOLE BLOOD GENOMIC DNA EXTRACTION
MAIN PROTOCOL

Sample

In a 1.5 mL microcentrifuge tube, add:
a. PKD Solution: 25 µL
b. Whole Blood: 200 µL
c. LBD Solution: 250 µL

i. Add a–b–c in the given order.
ii. Use blood collected in an EDTA tube.

Lysis

Vortex to mix until all contents are homogeneous (15 seconds).

Incubation

Incubate at 56°C for 5 minutes.

Add Ethanol

Add absolute ethanol (>96%): 250 µL.

i. Mix the lysate by pipetting.
ii. Transfer all contents to the spin column.

Bind

Centrifuge at 8,000 x g for 1 minute.
Discard the flow-through and change the collection tube.

Wash 1

Add 500 µL WBD-1 Solution.
Centrifuge at 8,000 x g for 1 minute.
Discard the flow-through and change the collection tube.

Wash 2

Add 500 µL WBD-2 Solution.
Centrifuge at 8,000 x g for 1 minute.
Discard the flow-through and change the collection tube.

Dry Spin

Centrifuge at 8,000 x g for 2 minutes.
Transfer the column to a clean microcentrifuge tube for elution.

Elute

Add 50–100 µL EBD Solution to the center of the membrane.
Incubate at room temperature for 1 minute.

Final Spin

Centrifuge at 8,000 x g for 1 minute.

Genomic DNA

Purified genomic DNA is ready for use in PCR, qPCR, Southern blot, and other enzymatic applications.

All centrifugation steps: 8,000 x g (approx. 1 minute = ~10,000 rpm, depending on rotor).

KIT COMPONENTS

Whole Blood Genomic DNA Cat. No.: OttoP9510	250 Tests OttoP9510-250	100 Tests OttoP9510-100	50 Tests OttoP9510-50
Lysis Solution (LBD)	70 mL	27 mL	14 mL
Proteinase K Solution (PKD)	5 × 1.25 mL	2 × 1.25 mL	1.25 mL
Wash Solution 1 (WBD-1)	85 mL	34 mL	17 mL
Wash Solution 2 (WBD-2)	85 mL	33 mL	17 mL
Spin Columns and Collection Tubes	250	100	50
Elution Solution (EBD)	25 mL	15 mL	15 mL

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