Cat.NO. GERC -101074-100

Store the kit at 15 to 25℃

Genorise Whole Blood DNA 15min Kit 100

For DNA purification from whole blood

For Research Use only

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Whole Blood Genomic DNA Purification

Step	Procedure
1 Prepare Buffer GL	Add 500μL Buffer GL
2 Lyse sample	Add 200-250μL whole blood Vortex 30 Sec
3 protein precipitation	Add 100μL Buffer GPP Vortex 30 Sec
4 protein precipitation	12,000×g 5 min
5 Bind DNA 6 Wash Silica Membrane 7 Wash Silica Membrane 8 Wash Silica Membrane	12,000×g 1 min 1 Wash 500μL GW1 2 Wash 500μL GW2 ; 700μL GW2 12,000×g 1 min
9 Dry Silica Membrane	12,000×g 2 min
10 Elute Highly Pure DNA	50μL Buffer GE(70℃) RT 1 min 12,000×g 1 min

Kit Contents

Genorise Whole Blood DNA mini Kit	100
Cat No.	GERC-101074-100
DNA binding columns	100
Collection Tubes	100
Buffer GL	55ml
Buffer GPP	12ml
Buffer GW1	38ml +24ml ethanol
Buffer GW2	38ml +92ml ethanol
Buffer GE	25ml

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6. Troubleshooting

NO or poor DNA yield

Incomplete lysis

- Low WBC content in blood sample
- Inefficient lysis with Buffer GL
- Inefficient mixing with Buffer GPP
- DNA not efficiently eluted
- Blood coagulation

②**Ethanol not added to GW1and Buffer GW2**

- Add absolute ethanol to the buffers before using.
- After adding ethanol, mix the buffers well and store at +15 to +25℃.
- Always mark GW1vial and Buffer GW2 vial to indicate whether ethanol has been added or not.

Purified DNA sample cannot easily be loaded into the well of an agarose gel, but instead "pops out" of the well as it is loaded.

Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.

- .After the last wash step, make certain flowthrough solution containing Wash Buffer does not contact the bottom of the Filter Tube.
- If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and re-centrifuge for 30 seconds.

Eluate from blood is still slightly colored.

Incomplete wash.

- Wash Filter Tube until flowthrough is colorless.
- Repeat purification by using 200 μL eluate from the first purification round as starting material; add 400 μL Buffer GL, mix well, then add 200 μL ethanol（95-100%）.
- Follow corresponding procedure, starting with the application of the sample to the DNA binding column.

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1. Introduction

- **Whole Blood DNA Mini Kit** is designed for rapid,small-scale preparation of highly pure genomic DNA form Animal Blood cells.The purified DNA can be used directly for PCR,Southern blotting,or any kind of enzymatic reactions.
- The kit allows purification of up to 12 ug of pure genomic DNA with an A260/A280 ratio between 1.7 and 1.9,and is up to 50kb in size,with fragments of 30 kb predominating.The Kit procedure also efficiently recovers DNA fragments as small as 100 bp.
- Blood genomic DNA is directly isolated from the white blood cell (WBC) component of whole blood, without the need to remove the red blood cells (RBCs) in advance. This kit can also be used to extract DNA from dried blood.
- Buffer GL will efficiently destroy any bacteria and virus present in the blood sample, there by reducing the potential risk of these infectious agents.
- This kit does not require cell lysis proteinase K.

2.Storage

- DNA binding columns,and all buffers should be stored dry ,at room temperature(15-25°C) and are stable for 1 year under these conditions.

3. Equipment and Reagent to Be Supplied by User

Refer to the list below for additional reagents and equipment required for all isolation procedures:

- Absolute ethanol（95-100%）for **GW1**and **Buffer GW2**
- RNase treatment (optional): ●RNase, DNase-free
- Heating block or water bath.
- PBS

4. DNA Purification Protocols

4.1 Sample Material

- 200-250 μL mammalian whole blood
- ≤10μL avian blood or amphibian blood

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4.2 Purification of Total DNA from Whole Blood

①To a nuclease free 1.5 ml microcentrifuge tube
●Add 500 μL of **Buffer GL**.

▲ Before starting the purification reaction,
warm up the Elution Buffer(GE or H_2O) to 70°C.

500 μL Buffer GL

②Add 200-250 μl of anti-coagulated whole blood. Close the cap of the Microfuge tube and mix by vortexing at top speed for 10-30 seconds.

●If your sample volume is less than 200 μL, the sample volume should be expanded with PBS.
●If using avian blood or amphibian blood, combine <10 μl of blood with 200 μl of PBS.

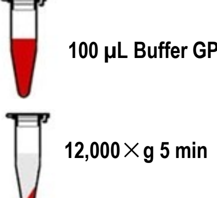
●To extract genomic DNA from clotted or dried blood, place the sample in a mortar (ambient temperature) and add 200 μl of 20mM Tris, 10mM EDTA, pH 8.5. Grind rapidly for 30 seconds to disperse the sample.
Add 500 μl of Buffer GL pre-heated to 50°C and grind briefly or pipette to dissolve the sample. Transfer the sample to a 1.5 ml Microfuge tube with a transfer pipette or other device. Vortex for 10 seconds to further dissolve the dried or clotted blood.

200 μL Blood

③Add 100 μl of Buffer GPP and mix by **vortexing** at top speed for 30 seconds.

④Centrifuge
12,000×g for 5-10 minutes at ambient temperature to pellet cellular debris.

⑤●Insert one DNA binding column in one Collection Tube.
●Pipet the sample into the upper buffer reservoir of the DNA binding column.
●Insert the DNA binding column assembly into a standard tabletop centrifuge.
●Centrifuge 1 min at 12,000×g.



100 μL Buffer GPP

12,000×g 5 min

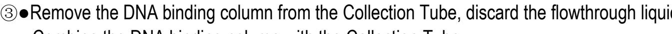
4.3 Protocol for Washing and Elution

①After centrifugation:
●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
●Combine the DNA binding column with the Collection Tube.
●Add 500 μL **Buffer GW1**. Removal Buffer to the upper reservoir of the Filter Tube.
●Centrifuge 1 min at 12,000×g.

500ul GW1
12,000×g 1min

②●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
●Combine the DNA binding column with the Collection Tube.
●Add 500μL **Buffer GW2** to the upper reservoir of the DNA binding column.
●Centrifuge 1 min at 12,000×g and discard the flowthrough.

500ul GW2
12,000×g 1min



③●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
●Combine the DNA binding column with the Collection Tube.
●Add 700 μL **Buffer GW2** to the upper reservoir of the DNA binding column.
●Centrifuge 1 min at 12,000×g and discard the flowthrough.

700ul GW2
12,000×g 1min

④After discarding the flowthrough liquid:
●Centrifuge the DNA binding column for additional 1min at full speed.
●Discard the Collection Tube.
●Insert the DNA binding column into a clean, sterile 1.5 ml microcentrifuge tube.
●Dry the DNA binding column at Room temperature for two minutes
▲Ensure removal of residual Wash Buffer.

12,000×g 1min

⑤To elute the DNA:
●Add 200 μL prewarmed **Buffer GE** to the upper reservoir of the DNA binding column.
●Incubate the DNA binding column for 2minutes at at Room temperature.
●Centrifuge the tube assembly for 1 min at 12,000×g 1min.
▲Elution with 50μL (instead of 200μL) increases the final DNA concentration in the eluate , but also decreases the overall DNA yield.
▲Warm up the **Buffer GE** to 70℃ increases the final DNA concentration in the eluate.
▲If you wish to remove RNA from the eluted DNA treat your sample as follows:
Add to the RNase A 2μL (10mg/ml) and incubate as appropriate(37℃,15min).

12,000×g 1min

⑥The microcentrifuge tube now contains the eluted DNA.
Either use the eluted DNA directly or store the eluted DNA at +2 to +8℃ or 15 to 25℃ for later analysis.

5. Results

●**Purity:** Purified nucleic acids are free of other cellular components and DNA polymerase inhibitors.
▲RNA can be removed from purified DNA with an optional RNase digestion.

Expected Yield Variable depending on sample type. The table below shows experimental results:

Elution with 50μL Buffer GE

Sample	Yield(ug) Total nucleic acids	OD260/280	Conc(ng/ul)
50ul Blood	1.2	1.96	24
100ul Blood	2.6	2.08	52
200ul Blood	6.2	2.01	124
250ul Blood	7.0	1.95	140