



**Cat.NO. GERC-101075-500**










Store the kit at 15 to 25°C

## **Genorise Whole Blood DNA 15min Kit 500**

For DNA purification from whole blood

For Research Use only

## Whole Blood Genomic DNA Purification

1 Prepare Buffer GL		Add 500 $\mu$ L Buffer GL
2 Lyse sample		Add 200-250 $\mu$ L whole blood Vortex 30 Sec
3 protein precipitation		Add 100 $\mu$ L Buffer GPP Vortex 30 Sec
4 protein precipitation		12,000 $\times$ g 5 min
5 Bind DNA		12,000 $\times$ g 1 min
6 Wash Silica Membrane		1 Wash 500 $\mu$ L GW1
7 Wash Silica Membrane		2 Wash 500 $\mu$ L GW2 ; 700 $\mu$ L GW2
8 Wash Silica Membrane		12,000 $\times$ g 1 min
9 Dry Silica Membrane		12,000 $\times$ g 2 min
10 Elute Highly Pure DNA	 	50 $\mu$ L Buffer GE(70 $^{\circ}$ C) RT 1 min 12,000 $\times$ g 1 min

## Kit Contents

Genorise Whole Blood DNA mini Kit	500
Cat No.	GERC-101075-500
DNA binding columns	500
Collection Tubes	500
Buffer GL	100ml $\times$ 3
Buffer GPP	60ml
Buffer GW1	80ml +50ml ethanol $\times$ 2
Buffer GW2	38ml +92ml ethanol $\times$ 5
Buffer GE	50ml

## 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 GW1 and Buffer GW2

- Add absolute ethanol to the buffers before using.
- After adding ethanol, mix the buffers well and store at +15 to +25°C.
- Always mark GW1 vial 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 from 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 µg 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, thereby 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

## 4.2 Purification of Total DNA from Whole Blood

① To a nuclease free 1.5 ml microcentrifuge tube

- Add 500  $\mu$ L of **Buffer GL**.

- ▲ Before starting the purification reaction, warm up the Elution Buffer(GE or H<sub>2</sub>O) to 70°C.



② Add 200-250  $\mu$ l 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  $\mu$ L, the sample volume should be expanded with PBS.
- If using avian blood or amphibian blood, combine <10  $\mu$ l of blood with 200  $\mu$ l of PBS.



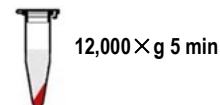
- To extract genomic DNA from clotted or dried blood, place the sample in a mortar (ambient temperature) and add 200  $\mu$ l of 20mM Tris, 10mM EDTA, pH 8.5. Grind rapidly for 30 seconds to disperse the sample. Add 500  $\mu$ l of Buffer GL pre-heated to 50°C and grind briefly or pipette to dissolve 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.

③ Add 100  $\mu$ l of Buffer GPP and mix by **vortexing** at top speed for 30 seconds.



④ Centrifuge

- 12,000  $\times$  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  $\times$ g.

## 4.3 Protocol for Washing and Elution

① After 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  $\mu$ L **Buffer GW1**. Removal Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 12,000  $\times$ g.



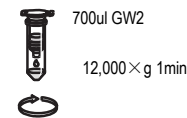
② ● Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.

- Combine the DNA binding column with the Collection Tube.
- Add 500  $\mu$ L **Buffer GW2** to the upper reservoir of the DNA binding column.
- Centrifuge 1 min at 12,000  $\times$ g and discard the flowthrough.



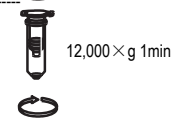
③ ● Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.

- Combine the DNA binding column with the Collection Tube.
- Add 700  $\mu$ L **Buffer GW2** to the upper reservoir of the DNA binding column.
- Centrifuge 1 min at 12,000  $\times$ g and discard the flowthrough.



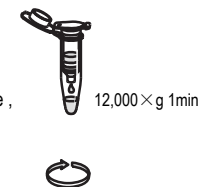
④ After discarding the flowthrough liquid:

- 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.



⑤ To elute the DNA:

- Add 200  $\mu$ L 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  $\times$  g 1min.
- ▲ Elution with 50 $\mu$ L (instead of 200 $\mu$ L) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield.
- ▲ Warm up the **Buffer GE** to 70°C 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 2 $\mu$ L (10mg/ml) and incubate as appropriate(37°C, 15min).



⑥ The microcentrifuge tube now contains the eluted DNA.

Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or 15 to 25°C 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 50 $\mu$ L 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