Genomic DNA Midi Kit (Blood) Protocol

For research use only

Catalogue Number

GDI002, GDI100

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
2. Prepare Phosphate Buffered Saline (PBS, pH7.2).
3. Yield and quality of DNA will be higher when fresh samples are used.
4. Optionally prepare RNase A (50 mg/ml) for RNA-free DNA. However, residual RNA will not affect PCR.

3 ml Blood Protocol Procedure

1. Blood Sample Preparation

Transfer **150 µl of Protease to the bottom of a 15 ml centrifuge tube** then add 1-3 ml of whole blood.

Note: If the blood volume is less than 3 ml, adjust the volume to 3 ml with PBS.

2. Cell Lysis

Add **3 ml of GSB Buffer** and mix by inverting the tube 10 times then by shaking the tube vigorously.
NOTE: Do Not add Protease directly to GSB Buffer before use.

Incubate at 60ºC for at least 20 minutes, inverting the tube every 5 minutes. During incubation, transfer required volume of Elution Buffer (200µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).

Optional RNA Removal Step

For RNA-free gDNA, following GSB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by vortex. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

3. DNA Binding

Add **3 ml of absolute ethanol** to the sample lysate and mix **IMMEDIATELY** by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette.

Centrifuge Protocol

Connect the **GS Column to the Column Extension Tube**. Pressing the column lid down and slide the assembly into a clean 50 ml centrifuge tube. Transfer all of the sample mixture into the assembly and centrifuge at 1,500 x g for 2 minutes. Discard the flow-through. Disconnect the column and place it in a 2 ml Collection Tube.

Vacuum Protocol

Connect the **GS Column to the Column Extension Tube**. Connect the assembly to a vacuum manifold. Transfer the entire sample mixture into the assembly. Apply vacuum at 15 inches Hg until the sample passes through the column. Switch off the vacuum. Disconnect the column and place it in a 2 ml Collection Tube.

4. Wash

Add **400 µl of W1 Buffer to the GS Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the GS Column again. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

5. Elution

Transfer the dry GS Column to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**, TE Buffer or water into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute purified DNA.

Kit Components

Component	GDI002	GDI100
GSB Buffer	15 ml	155 ml x 2
W1 Buffer	2 ml	45 ml
Wash Buffer [1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)
Protease [2]	320 µl	5.5 ml x 3
Elution Buffer	1 ml	30 ml
GS Column Extension Tubes	2	100
Column Extension Tubes	2	100
2 ml Collection Tubes	2	100

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. Protease is shipped at room temperature and should be stored at 2-8ºC for up to 6 months.

Storage: dry at room temperature (15-25ºC)