gDNA Maxi Kit (Blood/Cell) Quick Protocol

For research use only

Catalogue Number

GDM002, GDM010, GDM025

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

IMPORTANT BEFORE USE!

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

2. 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.

1. Sample Preparation

Fresh or Frozen Blood

Transfer **500 µl of Protease** to the bottom of a 50 ml centrifuge tube. Add **3-10 ml of whole blood**. If the blood volume is less than 10 ml, add the appropriate volume of PBS.

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add **0.10-0.25% Trypsin in PBS**. Once cells detach add medium then transfer to a 50 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer **cells (up to 1 x 10^8)** to a 50 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant then resuspend cells in **10 ml of PBS**. Add **500 µl of Protease** into the 50 ml centrifuge tube then mix by shaking briefly.

2. Lysis

Add **10 ml of GB Buffer** into the centrifuge tube and mix the sample thoroughly by inverting the tube 10 times, followed by vigorous shaking. It is essential that the sample and GB Buffer are mixed thoroughly to yield a homogenous solution. DO NOT add protease directly to GB Buffer before use. Incubate the sample mixture at 60ºC for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, pre-heat the required volume of Elution Buffer (2 ml/ sample) to 60ºC for step 5 DNA elution.

Optional Step: RNA Degradation

Following 60ºC incubation, add 50 µl of RNase A (10 mg/ml) to the clear lysate and shake vigorously. Incubate at room temperature for 10 minutes.

3. DNA Binding

Add **10 ml of absolute ethanol** to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Transfer **15 ml of the sample mixture** (including any precipitate) to the **GD Maxi Column** in Collection Tube. Close the cap and centrifuge at 3,000 x g for 3 minutes then discard the flow-through. Place the **GD Maxi Column** back in the Collection Tube then transfer the remaining mixture to the **GD Maxi Column**. Close the cap and centrifuge at 3,000 x g for 3 minutes then discard the flow-through.

4. Wash

Place the **GD Maxi Column** back in the Collection Tube and add **4 ml of W1 Buffer** into the **GD Maxi Column**. Close the cap and centrifuge at 3,000 x g for 2 minutes then discard the flow-through. Place the **GD Maxi Column** back in the Collection Tube. Add **12 ml of Wash Buffer** (make sure ethanol was added) to the **GD Maxi Column** then let stand for 2 minutes. Close the cap and centrifuge at 3,000 x g for 2 minutes then discard the flow-through. Place the **GD Maxi Column** back in the Collection Tube then centrifuge at 3,000 x g for 10 minutes to dry the column matrix.

5. Elution

Transfer the dried **GD Maxi Column** to a new Collection Tube. Add **500 µl-1 ml of pre-heated Elution Buffer**[1], TE[2] or water[3] into the CENTER of the column matrix. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA.

For maximum DNA concentration: Reload the eluate containing the DNA into the center of the column matrix. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA again.

For maximum DNA yield: Repeat the elution step by adding 500 µl-1 ml of pre-heated Elution Buffer into the center of the column matrix again. Incubate at room temperature for 3 minutes then centrifuge at 3,000 x g for 5 minutes to elute the purified DNA. The total elution volume is approximately 1-2 ml.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the CENTER of the column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the CENTER of the column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the CENTER of the column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Components

Component	GDM002	GDM010	GDM025
GB Buffer	25 ml	120 ml	280 ml
Protease[1]	1.1 ml	5.5 ml	6.5 ml x 2
W1 Buffer	10 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	25 ml x 1 (100 ml) 50 ml x 1 (200 ml x 1)
Elution Buffer	6 ml	30 ml	60 ml
GD Maxi Columns in Collection Tube	2	10	25
Collection Tube with Cap	2	10	25

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

[2]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.

Storage

Dry at room temperature (15-25ºC). Protease is shipped at room temperature and should be stored at 2-8ºC for up to 6 months.