GSure® Blood & Cell Culture DNA Mini Kit

#G4624A 50 preparations

Store at Room Temperature

Procedure:

1. Pellet down blood cells/ cultured cells in microfuge tube by centrifugation with a tabletop centrifuge at 300Xg speed for 5 min.
2. Resuspend harvested cells in 250µl Buffer GDBC1. Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to re-suspend the cells.
3. Incubate the tube at 70^0C for 15 minutes and vortex after every 2 minutes. Color of the suspended cells (for blood) may turn dark brown at this time.
4. Add 250µl Buffer GDBC2 and mix by inverting the tube 4–6 times. Place the tube at 70^0C again for another 15 minutes. Color of the suspended cells (for blood) may turn dark brown at this time also.
5. Add 350µl Buffer GDBC3 and invert the tube immediately. Shake vigorously to mix the solutions, **DO NOT VORTEX AT THIS STAGE**. Vortex may cause shearing of genomic DNA.
6. Centrifuge for 10 minutes at 13000rpm (~8500g) in a table-top microcentrifuge. A compact dark brown pellet will form (for blood).
7. Apply supernatant to the Gmini Spin Column by decanting or pipetting. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog Gmini spin Column thus lowering the DNA yield.
8. Centrifuge at 13000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
9. Wash Gmini Spin Column by adding 600µl Membrane Wash Buffer and centrifuging for 30–60 s as previously.
10. Discard the flow-through.
11. Repeat washing step.
12. Discard the flow-through, and centrifuge for an additional 2 minutes to remove residual wash buffer from membrane.

> ***This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified DNA may inhibit successive enzymatic reactions.***

13. Place the Gmini Spin Column in a clean 1.5ml microcentrifuge tube (not provided).

    To elute DNA, add 50µl Nuclease-free Water (provided) to the center of each Gmini Spin Column, let it stand for 1 minute, and centrifuge for 1 minute at maximum speed (~8500Xg) on a table top microcentrifuge.
14. Discard the column and collect the eluted DNA present in microcentrifuge tube.

- If require, increase the volume of GDBC1, GDBC2 and GDBC3 accordingly.
- **If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.**

Kit Contents:

GDBC1 Buffer	**: 15ml**
GDBC2 Buffer	**: 15ml**
GDBC3 Buffer	**: 20ml**
Membrane Wash Buffer	**: 30ml**
Gmini-Spin-Column	**: 50pcs.**
Nuclease free water	**: 3 ml**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 45ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap