GSure® Blood DNA Mini Kit

#G4626 250 preparations

Store at Room Temperature.

For long term storage of RBC Lysis Buffer store at 4° C

Procedure:

Removal of RBC:

To remove RBC from total blood, collect fresh or stored blood **(minimum 0.2 ml to maximum 1 ml)** in a fresh tube. **Add 5X sample volume of RBCL Buffer**, mix thoroughly by vortexing, incubate the tube in ice for 15min. During incubation, vortex the tube for at least two times after regular intervals. Centrifuge the sample at 400xg for 10 minutes; a whitish pellet should form at the bottom of the tube. Remove supernatant by pipetting.
Caution: while removing the supernatant, pellet should not be disturbed.

Add 2X sample volume of RBCL Buffer again in the cell pellet, mix by vortexing. While mixing, cell pellet should be dislodged from the tube. Incubate again in ice for another 15min, vortex twice intermittently in a regular time interval. Centrifuge at 400xg for 10 minutes. Remove supernatant completely and use harvested white colored cell population in pellet as sample for DNA isolation.

DNA isolation:

1. Resuspend the pellet in 250 µl of GDBL1 by vigorous vortexing (tap vortexing for better efficacy). Incubate the tube at 70^{0}C for 15 minutes and vortex after every 2 minutes.
2. Add 250µl Buffer GDBL2 and mix by inverting the tube 4–6 times. Place the tube at 70^{0}C again for another 15 minutes.
3. Add 350µl Buffer GDBL3 and invert the tube immediately. Shake vigorously to mix the solutions. **DO NOT VORTEX AT THIS STAGE**. Vortex may cause shearing of genomic DNA.
4. Centrifuge for 10 minutes at 10000Xg in a table-top microcentrifuge.
5. Apply the 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.
6. Centrifuge at 8500Xg for 30–60 s. Discard the flow-through.
7. Wash GMini Spin Column by adding 600µl Membrane Wash Buffer* and centrifuging for 30–60 s as previously.
8. Discard the flow-through.
9. Repeat washing step.
10. 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.

11. Place the GMini SpinColumn in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50 µl Nuclease-free Water(provided) to the center of each GMini Spin Column, let stand for 1 minute, and centrifuge for 1 minute at maximum speed (~8500Xg) on a table top microcentrifuge.
12. Discard the column and collect the eluted DNA present in microcentrifuge tube.

- If required, increase the volume of GDBL1, GDBL2 and GDBL3 accordingly.
- **If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.**

Kit Contents:

GDBL1 Buffer	:	75ml
GDBL2 Buffer	:	75ml
GDBL3 Buffer	:	100ml
Membrane Wash Buffer	:	75ml x 2
GMini Spin Column	:	50 pcs X 5
RBCL Buffer	:	400 ml X 5
Nuclease-free Water	:	15 ml

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 112.5 ml of absolute ethanol (molecular biology grade) with the provided buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.