GSure® Bacterial Genomic DNA Isolation Kit

#G46211 **50 reparations**

Store at Room Temperature

Procedure:

1. Pellet down overnight grown bacterial cells in a microfuge tube by centrifugation, Discard the supernatant and add 250 µl GDB1 to cell pellet. Resuspend vigorously till no clumps are visible.
2. Incubate the tube at 70^{0}C for 15 min and vortex after every 2 min.
3. Add 250 µl Buffer GDB2 and mix by inverting the tube 4–6 times. Place the tube at 70^{0}C again for another 15 minutes.
4. Add 350 µl Buffer GDB3 and invert the tube immediately. Shake vigorously to mix the solutions, **DO NOT VORTEX AT THIS STAGE.** Vortexing may cause shearing of genomic DNA.
5. Centrifuge for 10 min at 13,000 rpm (~8500xg) in a table-top microcentrifuge.
6. 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.
7. Centrifuge at 13,000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
8. Wash GMini Spin Column by adding 600µl Membrane Wash Buffer* and centrifuging for 30–60 s as previously.
9. Discard the flow-through.
10. Repeat washing step.
11. 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.***

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

- If required, increase the volume of GDB1, GDB2 and GDB3 accordingly.
- **If any sediments found in any of the buffers, warm the containers at 50°C until it dissolves.**

Kit Contents:

GDB1 Buffer	: 15ml
GDB2 Buffer	: 15ml
GDB3 Buffer	: 20ml
Membrane Wash Buffer	: 30ml
Spin Columns	: 50 pcs.
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. After ethanol addition, tighten the cap properly after each use.