GSure® DOGMA Kit for Bacteria

One kit for DNA-RNA-Protein Isolation

#GD1001 50 preparations

Store at Room Temperature

Procedure:

1. Pellet down 500 µl of overnight grown bacterial cells in a microfuge tube by centrifugation.
2. Resuspend harvested cells in 250 µl Buffer GDGB1. Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to resuspend the cells.
3. Incubate the tube at 70^{0}C for 15 min and vortex after every 2 min.
4. Centrifuge the sample at maximum speed (10000Xg) for 10min in a table top centrifuge at room temperature.
5. Collect 250 µl of clear supernatant in a fresh microfuge tube.
6. Add 250 µl Buffer GDGB2 with the collected supernatant and mix by inverting the tube 4-6 times.
7. Add 350 µl Buffer GDGB3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
8. Take one ***GMini DNA binding column (white colored)*** and load the whole solution from previous step on column.
9. Centrifuge for 1min at maximum speed (10000Xg) in a table top centrifuge at room temperature.
10. Collect the flow through in a fresh 2ml microfuge tube. Do not discard the column, it contains the isolated DNA.
11. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
12. Apply 600ul of the isopropanol-added flowthrough in ***GMini Chrom-Column (Column specified for RNA binding)*** by pipetting.
13. Centrifuge at 10000xg for 30–60 s. **Collect the flow through in a fresh 1.5ml microfuge tube**. **Do not discard the column, it contains the isolated RNA.**
14. Apply remaining 600ul of the isopropanol-added flowthrough in same ***GMini Chrom-Column (Column specified for RNA binding)*** again by pipetting.
15. Centrifuge at 10000xg for 30–60 s. **Collect the flow through in another fresh 1.5ml microfuge tube**. **Do not discard the column, it contains the isolated RNA.**
16. Leave the collected flowthrough at room temperature. This flowthrough could be stored at room temperature for overnight. **DONOT STORE THE FLOWTHROUGH AT COLD TEMPERATURE.**
17. Wash both ***GMini DNA binding column and GMini Chrom-Column*** by adding 600µl Membrane Wash Buffer and centrifuging at (10000Xg) for 30–60s. Discard the flow-through.
18. Repeat washing step.
19. Discard the flow-through, and centrifuge at 10000Xg, for an additional 2 min to remove residual wash buffer from membranes.

    This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified DNA/RNA may inhibit downstream application.
20. Place the ***GMini DNA binding column and GMini Chrom-Column*** in two separate clean 1.5 ml microcentrifuge tubes (not provided). To elute DNA and RNA, add 50 µl Nuclease-free Water to the center of ***GMini DNA binding column and GMini Chrom-Column***, let stand for 1 min, and centrifuge for 1 min at maximum speed (~8500Xg) on a table top microcentrifuge at room temperature.
21. Discard the columns and collect the eluted DNA* and RNA# present in microcentrifuge tubes.
22. Now, take any one of the collected flowthrough and add 500ul of deionized water in the tube.

23. Add 500ul of chloroform in the same tube.

24. Vortex vigorously for 5-10 sec.

25. Centrifuge the tube at maximum speed in a table top miocrocentrifuge (~10000xg) for 2min.

26. Carefully remove the tube from centrifuge, discard the clear aquous phase (upper phase) by pipetting.

> **N.B.: At the interphase, a whitish interphase containing protein will be visible. Remove the upper aquous phase by not disturbing the interphase.**

27. Add 500ul of absolute ethanol (room temperature stored) in the tube and vortex vigorously.

28. Centrifuge the tube at maximum speed in a table top miocrocentrifuge (~10000xg) for 2min.

29. Discard the supernatant, a whitish protein pellet will form at the bottom of the tube.

> **Note: Protein pellet forms at the bottom of the tube is very loosly bound, carefully remove the supernatant by not disturbing the pellet.**

30. Place the protein pellet at 90^0C heat block, opening the tube cap. This will evaporate the residual alcohol present in the protein pellet.

31. Once the pellet is dried completely, add 20ul of protein resuspension buffer in the tube and vortex vigorously again for 15-30 sec.

32. Place the tube in heat block at 90^0C for 10 min to completely resolubilize the protein pellet.

> **Note: If require, add 10-20ul more redissolving buffer to dissolve the protein sample. If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.**

Kit Contents:

GDGB1 Buffer	**:**	**15ml**
GDGB2 Buffer	**:**	**15ml**
GDGB3 Buffer	**:**	**20ml**
Membrane Wash Buffer	**:**	**60ml**
GMini DNA binding Column	**:**	**50 pcs.**
GMini Chrom-Column	**:**	**50 pcs.**
Nulcease free Water	**:**	**3ml X 2**
Protein Resuspension Buffer	**:**	**3ml X 2**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 90ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.

* Depending of cell type, the isolated DNA may contain contaminating RNA. In such cases, it is advisable to treat the DNA to DNase-free RNaseA digestion prior to downstream applications.

The isolated RNA may contain contaminating DNA. In such cases, it is advisable to treat the RNA to RNase-free DNase digestion prior to downstream applications.