GSure® DOGMA Kit for Cultured Cell

One kit for DNA-RNA-Protein Isolation

#GD1003 50 preparations

Store at Room Temperature

Procedure:

1. Pellet down ~10^6 cells in microfuge tube by centrifugation with a tabletop centrifuge at 300Xg speed for 5 minutes. Wash twice with PBS.
2. Resuspend harvested cells in 250µl Buffer GDGC1. 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 minutes and vortex after every 2 minutes.
4. Centrifuge the sample at maximum speed (10000Xg) for 10minutes in a table top centrifuge at room temperature.
5. Collect the clear supernatant in a fresh microfuge tube.
6. Add 250µl Buffer GDGC2 with the collected supernatant and mix by inverting the tube 4-6 times.
7. Add 350µl Buffer GDGC3 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 minute, and centrifuge for 1 minute 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 but not disturbing the pellet.

30. Place the protein pellet at 90^{0}C 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 minutes to completely resolubilize the protein pellet.

Note: If require, add 10-20ul more redissolving buffer to dissolve the protein sample.

Note: If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.

Kit Contents:

GDGC1 Buffer : 15ml
GDGC2 Buffer : 15ml
GDGC3 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.