GSure® DOGMA KIT FOR BLOOD

One kit for DNA-RNA-Protein Isolation

#GD1005A **20 preparations**

Storage: Room Temperature

Procedure:

Sample preparation:

Starting with 0.2ml-1ml of blood sample **GSure® DOGMA KIT FOR BLOOD** can isolate DNA, RNA and protein from 1ml of blood sample. Sample kit provides RBCL Buffer which is sufficient for isolation from upto 1ml of blood). Total blood sample contains two major components, blood cells and blood plasma. Blood contains around 50% Red Blood Cells (RBC). Mature Red Blood Cells are anucleated. Leucocytes contribute only 1% population of total blood. These leucocytes are the potential source of nucleic acid. High population of RBC is a major hindrance to isolate good quality of total RNA from blood. Thus, removal of RBC from total blood sample prior to total RNA isolation is an essential step.

GSure® Blood DOGMA kit comes with **RBCL Buffer** which selectively lyses red blood cells leaving leucocytes intact. To remove RBC from total blood, collect fresh or stored blood in a fresh tube. Add 5X sample volume of RBCL Buffer, mix thoroughly by vortexing, and incubate the tube in ice for 15min. During incubation, vortex the tube for at least two times after regular interval. Centrifuge the sample at 400Xg for 10 minutes, a whitish cell 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 pelleted cell, mix by vortexing. While mixing, cell pellet should be dislodged from the tube. Incubate again in ice for another 15 minutes, vortex twice intermittently in a regular time interval. Centrifuge again the sample at 400xg for 10 minutes. Remove supernatant completely and use pelleted white colored cell population as sample for total RNA-DNA isolation.

1. Resuspend harvested blood cells in 250µl Buffer GDGBL1. Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to resuspend the cells.
2. Incubate the tube at 70^{0}C for 15 minutes and vortex after every 2 minutes.
3. Centrifuge the sample at maximum speed (10000Xg) for 10 minutes in a table top centrifuge at room temperature.
4. Collect 250µl of clear supernatant in a fresh microfuge tube.
5. Add 250µl Buffer GDGBL2 with the collected supernatant and mix by inverting the tube 4-6 times.
6. Add Add 350µl Buffer GDGBL3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
7. Take one ***GMini DNA binding column (white colored)*** and load the whole solution from step 6 on column.
8. Centrifuge for 1min at maximum speed (10000Xg) in a table top centrifuge at room temperature.
9. Collect the flow through in a fresh 2ml microfuge tube. Do not discard the column, it contains the isolated DNA.
10. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
11. Apply 600ul of the isopropanol-added flowthrough in ***GMini Chrom-Column (Column specified for RNA binding)*** by pipetting.
12. 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.**
13. Apply remaining 600ul of the isopropanol-added flowthrough in same ***GMini Chrom-Column (Column specified for RNA binding)*** again by pipetting.
14. 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.**

15. Leave the collected flowthrough at room temperature. This flowthrough could be stored at room temperature for overnight. **DONOT STORE THE FLOWTHROUGH AT COLD TEMPERATURE.**

16. 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.

17. Repeat washing step.

18. 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.**

19. 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.

20. Discard the columns and collect the eluted DNA and RNA present in microcentrifuge tubes.

21. Now, take any one of the collected flowthrough and add 500ul of deionized water in the tube.

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

23. Vortex vigorously for 5-10 sec.

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

25. 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.**

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

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

28. 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.**

29. 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.

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

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

Kit Contents:

GDGBL1 Buffer	: 6ml
GDGBL2 Buffer	: 6ml
GDGBL3 Buffer	: 8ml
Membrane Wash Buffer	: 24ml
GMini DNA binding Column	: 20 pcs.
GMini Chrom-Column	: 20 pcs.
Nulcease free Water	: 3ml
Protein Resuspension Buffer	: 3ml
RBCL Buffer	: 160 ml

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 36ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking. Tighten the cap properly after each use.