GSure® Ultra Nucleic Acid Isolation Kit (Blood)

#GRD1003 50 preparations

Store at Room Temperature

(For long term storage of RBC Lysis Buffer Store at 4°C)

Procedure:

Sample preparation:

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 DNA-RNA from blood. Thus, removal of RBC from total blood sample prior to total RNA isolation is an essential step. GSure® Ultra nucleic acid isolation kit for Blood **(for 0.2ml-1ml of sample)** comes with **RBCL Buffer** which selectively lyse 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 tubes for at least two times at regular interval. Centrifuge the sample at 400xg for 10 min, 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 15min, 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 DNA-RNA isolation.

1. Resuspend harvested blood cells in 250 µl Buffer GDRBL1. Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to resuspend the cells.
2. Incubate the tube at 70^0C for 15 min and vortex after every 2 min.
3. Centrifuge the sample at maximum speed (10000Xg) for 10min in a table top centrifuge at room temperature.
4. Collect the clear supernatant in a fresh microfuge tube.
5. Add 250 µl Buffer GDRBL2 with the collected supernatant and mix by inverting the tube 4-6 times.
6. Add 350 µl Buffer GDRBL3 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 the isopropanol added flowthrough in ***GMini Chrom-Column (Column specified for RNA binding)*** by decanting or pipetting.
12. Centrifuge at 10000xg for 30–60 s. Discard the flow-through.
13. Wash both ***GMini DNA binding column and GMini Chrom-Column*** by adding 600µl Membrane Wash Buffer and centrifuge for 30–60 s as previously.
14. Discard the flow-through.
15. Repeat washing step.
16. 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 RNA may inhibit downstream application.***

17. 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 of Nuclease free water (provided) to the center of ***GMini DNA binding column and GMini Chrom-Column*** respectively. Let it stand for 1 min, and centrifuge for 1 min at maximum speed (~8500Xg) on a table top microcentrifuge at room temperature.

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

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

Kit Contents:

GDRBL1 Buffer	: 15ml
GDRBL2 Buffer	: 15ml
GDRBL3 Buffer	: 20ml
Membrane Wash Buffer	: 60ml
GMini DNA binding Column:	50 pcs.
GMini Chrom-Column	: 50 pcs.
Nulcease free water	: 3ml x 2
RBCL Buffer	: 400 ml

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 90 ml 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.