GSure® Blood RNA Kit

#GR1002A 20 preparations

Store at Room Temperature

Protocol:

GSure® Blood RNA Isolation Kit can isolate total RNA from different amount of blood samples, differently stored blood sample and different forms of blood. Starting sample volume should be **minimum 0.2ml to maximum 1ml.**

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. This leucocyte 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 by lysis from total blood sample prior to total RNA isolation is an essential step. GSure™ Blood RNA isolation kit 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 tube for at least two times after 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 RNA isolation.

Streamlined Protocol:

1. Resuspend harvested blood cells in 250µl Buffer GRBL1. 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 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 the clear supernatant in a fresh microfuge tube.
5. Add 250µl Buffer GRBL2 with the collected flowthrough and mix by inverting the tube 4–6 times.
6. Add 350µl Buffer GRBL3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
7. Take one **pre purification column (white colored)** and load the whole solution from previous step on column.
8. Centrifuge for 1 minute at maximum speed (10000Xg) in a table top centrifuge at room temperature.
9. **Discard the column, not the flowthrough.** This flowthrough contains total RNA population.
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 Gmini Chrom-Column by adding 600µl Membrane Wash Buffer and centrifuging for 30–60 s as previously.
14. Discard the flow-through.
15. Repeat washing step.

16. 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 RNA may inhibit downstream application.***

17. Place the Gmini Chrom-Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute RNA, add 50 µl Nuclease-free Water to the center of each 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.

18. Discard the column and collect the eluted RNA present in microcentrifuge tube.

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

Kit Contents:

GRBL1 Buffer	**:**	**15ml**
GRBL2 Buffer	**:**	**15ml**
GRBL3 Buffer	**:**	**20ml**
Membrane Wash Buffer	**:**	**30ml**
Pre-purification Column	**:**	**50 pcs.**
GMini Chrom-Column	**:**	**50 pcs.**
Nulcease free water	**:**	**3ml**
RBCL Buffer	**:**	**400 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. Once ethanol added, tighten the cap properly after each use.