GSure® Bacterial RNA Kit

#GR1001 50 preparations

Store at Room Temperature

Procedure:

1. Pellet down 500µl of overnight grown cells in microfuge tube by centrifugation with a tabletop centrifuge at 3000Xg for 5 minutes. Completely remove the supernatant by aspiration with micropipette.
2. Resuspend harvested cells in 250µl Buffer GRB1. Resuspension should be done by vigorous vortexing; till no clumps are visible.
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 10 minutes in a table top centrifuge at room temperature.
5. Carefully collect the supernatant in a fresh microfuge tube.
6. Add 250µl Buffer GRB2 to the collected flowthrough and mix by inverting the tube 4–6 times.
7. Add 350µl Buffer GRB3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
8. Take one **Pre-purification column** and load the whole solution from previous step on column.
9. Centrifuge for 1 minute at maximum speed (10000Xg) in a table top centrifuge at room temperature.
10. Discard the column, **not the flowthrough.** This flowthrough contains total RNA population.
11. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
12. Apply the flowthrough added with isopropanol to **GMini Chrom Column (Column specified for RNA binding)** by decanting or pipetting.
13. Centrifuge at 10000xg for 30–60 s. Discard the flow-through.
14. Wash GMini Chrom Column by adding 600µl Membrane Wash Buffer* and centrifuging for 30–60 s as previously.
15. Discard the flow-through.
16. Repeat washing step.
17. 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 applications.***

18. Place the GMini Chrom Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute RNA, add 50µl Nuclease-free Water (provided) 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.
19. Discard the column and collect the eluted RNA present in microcentrifuge tube.

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

Kit Content:

GRB1 buffer	**:**	**15 ml.**
GRB2 buffer	**:**	**15 ml.**
GRB3 buffer	**:**	**20 ml**
Membrane Wash Buffer	**:**	**30 ml.**
Pre-purification Column	**:**	**50pcs.**
GMini Chrom Column	**:**	**50pcs.**
Nuclease-free Water	**:**	**3ml**

*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 is added, tighten the cap properly after each use.