

## GSure® Ultra Nucleic acid isolation kit (Bacteria)

#GRD1001

50 preparations

Store at Room Temperature

### Procedure:

1. Pellet down overnight grown bacterial cells in a microfuge tube by centrifugation, Discard the supernatant and add 250µl GDRB1 to cell pellet. Incubate the tube at 70°C for 15 min and vortex after every 2 min.
2. Centrifuge the sample at maximum speed (10000Xg) for 10min in a table top centrifuge at room temperature.
3. Collect the clear supernatant in a fresh microfuge tube.
4. Add 250µl Buffer GDRB2 and mix by inverting the tube 4–6 times.
5. Add 350µl Buffer GDRB3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
6. Take one **GMini DNA binding column (white colored)** and load the whole solution from step 6 on column.
7. Centrifuge for 1min at maximum speed (10000Xg) in a table top centrifuge at room temperature.
8. Collect the flow through in a fresh 2ml microfuge tube. **Do not discard the column, it contains the isolated DNA.**
9. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
10. Apply the isopropanol-added flowthrough in **GMini Chrom-Column (Column specified for RNA binding)** by decanting or pipetting.
11. Centrifuge at 10000xg for 30–60 s. Discard the flow-through.
12. Wash both **GMini DNA binding column and GMini Chrom-Column** by adding 700µl Buffer GDRBWB and centrifuging for 30–60 s as previously.
13. Discard the flow-through.
14. Repeat washing step.
15. 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.**

16. 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 (provided) to the center of **GMini DNA binding column and GMini Chrom-Column**, let stand

for 1 min, and centrifuge for 1 min at maximum speed (~8500Xg) on a table top microcentrifuge at room temperature.

17. 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:

GDRB1 Buffer	:	15ml
GDRB2 Buffer	:	15ml
GDRB3 Buffer	:	20ml
GDRBWB Buffer	:	60ml
GMini DNA binding Column	:	50 pcs.
GMini Chrom-Column	:	50 pcs.
Nulcease free Water	:	3ml X 2

### Reconstitution of GDRBWB:

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.