GSure® Ultra Nucleic Acid Isolation Kit (Tissue)

#GRD1005B 20 preparations

Store at Room Temperature

Procedure:

1. Take 25-30 mg of tissue sample (freshly collected/ stored in RNA later on collection afresh) in a fresh microfuge tube.
2. Wash tissue sample twice with 1X PBS thoroughly. Crush the tissue with a pipette-tip OR chop the tissue in smaller pieces.
3. Add 250 µl Buffer GDRT1. Incubate the tube at 70^{0}C for 15 min and vortex after every 2 min. Color may form depending on tissue type.
4. Centrifuge the sample at maximum speed (10000Xg) for 10min in a table top centrifuge at room temperature.
5. Collect the clear supernatant in a fresh microfuge tube.
6. Add 250 µl Buffer GDRT2 and mix by inverting the tube 4–6 times. Color may form depending on tissue type.
7. Add 350 µl Buffer GDRT3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
8. Take one ***GMini DNA binding column (white colored)*** and load the whole solution from step 7 on column.
9. Centrifuge for 1min at maximum speed (10000Xg) in a table top centrifuge at room temperature.
10. Collect the flow through in a fresh 2ml microfuge tube. **Do not discard the column, it contains the isolated DNA.**
11. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
12. Apply the isopropanol-added flowthrough in ***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 both ***Gmini DNA binding column and 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 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.***

18. 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-Colum*** respectively, let stand for 1 min, and centrifuge for 1 min at maximum speed (~8500Xg) on a table top microcentrifuge at room temperature.
19. 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:

GDRT1 Buffer	: 6ml
GDRT2 Buffer	**:** 6ml
GDRT3 Buffer	: 8ml
Membrane Wash Buffer	: 24ml
GMini DNA binding Column	: 20 pcs.
GMini Chrom-Column	: 20 pcs.
Nulcease free water	: 3ml

Reconstitution of Membrane Wash Buffer:

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.