GSure® Ultra Nucleic Acid Isolation Kit (Plant)

#GRD1004B 20 preparations

Store at Room Temperature

Procedure:

1. Take 20mg-30mg of freshly picked plant leaf. Rinse the leaf in RNaseZIP properly to remove contaminating RNase.
2. Take RNaseZIP treated plant tissue in a mortar, add 250 ul GDRP1 buffer to it. Add 250 ul either Part A or Part B. For most of the plant leaves, use part A. Part B is to be used when working with a plant type that produces high amount of secondary metabolites (e.g.: Pine, Bamboo, Tea, Neem etc). After addition of Part A or B, crush vigorously by pestle.
3. Collect 300µl-400µl of slurry in a fresh microfuge tube and vortex vigorously. Plant particle should also come with the buffer.
4. Incubate the tube at 70^0C for 15 minutes and vortex after every 5 min.
5. Centrifuge the sample at maximum speed (10000Xg) for 10min in a table top centrifuge at room temperature.
6. Collect 250 µl of the clear supernatant in a fresh microfuge tube.
7. Add 250 µl Buffer GDRP2 with the collected supernatant and mix by inverting the tube 4–6 times.
8. Add 350 µl Buffer GDRP3 and invert the tube immediately. Mix the buffer by inverting only. **DO NOT VORTEX TO MIX.**
9. Take one ***GMini DNA binding column (white colored)*** and load the whole solution from previous step on column.
10. Centrifuge for 1minute at maximum speed (10000Xg) in a table top centrifuge at room temperature.
11. Collect the flow through in a fresh 2ml microfuge tube. **Do not discard the column, it contains the isolated DNA.**
12. Add 600ul isopropanol in the collected flowthrough. Mix by inverting the tube several times.
13. Apply the isopropanol-added flowthrough in ***GMini Chrom-Column (Column specified for RNA binding)*** by decanting or pipetting.
14. Centrifuge at 10000xg for 30–60 s. Discard the flow-through.
15. Wash both ***GMini DNA binding column and GMini Chrom-Column*** by adding 600µl Membrane Wash Buffer and centrifuging for 30–60 s as previously.
16. Discard the flow-through.
17. Repeat washing step.
18. Discard the flow-through, and centrifuge at 10000Xg, for an additional 2 minutes 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.

19. 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 centre of ***GMini DNA binding column and 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.
20. 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:

GDRP1 Buffer	**: 6ml**
Part A	**: 6ml**
Part B	**: 6ml**
GDRP2 Buffer	**: 6ml**
GDRP3 Buffer	**: 8ml**
Membrane Wah Buffer	**: 24ml**
GMini DNA binding Column	**: 20pcs.**
GMini Chrom-Column	**: 20 pcs.**
Nulcease free Water	**: 3ml**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 36ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking.