GSure® Plant Mini Kit with WLN Buffer

#G4620W 50 preparations

Store at Room Temperature

Procedure:

1. Take ~25-50mg of plant tissue in a mortar, add 250 ul WLN 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.
2. Collect 300µl-400µl of slurry in a fresh microfuge tube and vortex vigorously. Plant particle should also come with the buffer.
3. Incubate the tube at 70^{0}C for 30 minutes and vortex after every 5 minutes.

Color of the suspended cells may turn dark green at this time.

4. After incubation step, centrifuge the tube at maximum speed (10,000xg) for 10 minutes, collect 250 ul of the clear supernatant in a fresh microfuge tube,
5. Add 250µl Buffer GDP2 and mix by inverting the tube 4–6 times. Color of the suspended cells may turn dark green at this time also.
6. Add 350µl Buffer GDP3 and invert the tube immediately. Shake vigorously to mix the solutions, **DO NOT VORTEX AT THIS STAGE**. Vortex may cause shearing of genomic DNA.
7. Apply the mixture to the GMini Spin Column by decanting or pipetting. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog GMini spin Column thus lowering the DNA yield.
8. Centrifuge at 13,000 rpm (~10000xg) for 30–60 s. Discard the flow-through.
9. Wash GMini Spin Column by adding 600µl membrane wash buffer and centrifuging for 30–60 s as previously.
10. Discard the flow-through.
11. Repeat washing step.
12. 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 DNA may inhibit successive enzymatic reactions.***

13. Place the GMini Spin Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50 µl Nuclease-free Water(provided) to the center of each GMini Spin

    Column, let stand for 1 minute, and centrifuge for 1 minute at maximum speed (~10000Xg) on a table top microcentrifuge.
14. Discard the column and collect the eluted DNA present in microcentrifuge tube.

- If required, increase the volume of WLN, GDP2 and GDP3 accordingly.
- **If any sediment found in any of the isolation buffers, warm the containers at 50°C until it dissolves.**

Kit Contents:

WLN Buffer	**: 15ml**
Part A	**: 15ml**
Part B	**: 15ml**
GDP2 Buffer	**: 15ml**
GDP3 Buffer	**: 20ml**
Membrane Wash Buffer	**: 30ml**
Spin Column	**: 50 pcs.**
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 added, tighten the cap properly after each use.