

DNA Isolation from Plant Tissue using the Nucleon PhytoPure DNA Extraction Kit

Breaking the cell wall:

1. Add a piece of dry ice to the mortar and pulverize it to a powder with the pestle (make sure both the mortar and pestle are very cold). Add about a gram of leaf tissue (1-2 small leaves) which has been frozen at -20°C .
2. Grind the tissue in the dry ice to yield a free-flowing powder.
3. Transfer the powder, using a chilled spatula, to a suitable polypropylene microcentrifuge tube.

Membrane lysis:

4. Add 600 μl of Reagent 1 (buffer solution), ensuring that all the reagent ingredients are fully dissolved (stir with a spatula, or cap the tube and tilt it *after the dry ice has completely melted*).
5. Add 200 μl of Reagent 2 (solution containing detergent).
6. Invert tube several times until a homogenous mixture is obtained.
7. Incubate at 65°C in a water bath for 10 minutes. Invert the tube several times every 2-3 minutes during incubation.
8. Place sample on ice for 20 minutes.

DNA extraction:

9. Remove sample from ice and add 500 μl of chloroform which has been stored at -20°C (do this in the hood).
10. Add 100 μl of Nucleon PhytoPure resin suspension (ensure that the resin is fully suspended by shaking vigorously immediately before use).
11. Manually agitate (invert the tube) the suspension for ten minutes at room temperature.
12. Centrifuge at 1300 rcf for 10 minutes.
13. Without disturbing the Nucleon resin suspension layer, use a pipette to transfer the upper DNA containing phase, above the brown resin layer, into a fresh centrifuge tube.

DNA precipitation:

14. Add an equal volume of cold isopropanol to the DNA phase in your tube.
15. Gently invert the tube until DNA precipitates (Precipitated DNA may be hooked out at this stage with a heat-sealed Pasteur pipette. Place the DNA into a clean tube containing 100 μl of sterile water.)
16. Centrifuge at a minimum of 4000 rcf for 5 minutes to pellet the DNA.
17. Wash the DNA pellet with cold 70% ethanol.
18. Centrifuge at a minimum of 4000 rcf for 5 minutes to pellet the DNA.
19. Discard the supernatant.
20. Air-dry the pellet for 10 minutes.
21. Resuspend the DNA in sterile water (100 μl).