E.Z.N.A.® Plant DNA DS Kit

Revision No: 2.1

omega BIO-TEK

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D2411-00	D2411-01	D2411-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
Homogenizer Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
CSPL Buffer	5 mL	40 mL	160 mL
RBB Buffer	5 mL	30 mL	120 mL
XP2 Binding Buffer	5 mL	30 mL	2 x 60 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	100 mL
Elution Buffer	2 mL	30 mL	30 mL
Proteinase K Solution	150 µL	1.5 mL	4.4 mL
RNase A (25 mg/ml)	30 µL	300 µL	1.2 mL

Supplied by user:

- Microcentrifuge capable of at least 12,000*g*
- Waterbath capable of 65°C
- Vortexer
- Nuclease-free 1.5 and 2 mL microcentrifuge tubes
- 100% isopropanol
- 100% ethanol
- Equipment and Materials required for the Sample Disruption Method selected (on reverse or see Pages 5-6 of the full manual available at www.omegabiotek.com)

Before starting:

- Prepare the HBC Buffer and DNA Wash Buffer according to the instructions on the bottles.
- Heat the Elution Buffer to 65°C.

DNA Extraction and Purification from Wet/Frozen/Dry Tissue Samples

LYSE

1. Prepare 10-50 mg wet/frozen tissue or 2-10 mg dry tissue in a 1.5 or 2 mL microcentrifuge tube/vial (not provided). See reverse for sample disruption techniques. For best results, use a commercial homogenizer if available.
2. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution. Vortex vigorously to mix. Make sure to disperse all clumps.
3. Incubate at 65°C for 30 minutes. Centrifuge at 12,000*g* for 3 minutes.
4. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.
5. Transfer 550 µL cleared supernatant to the Homogenizer Mini Column. Centrifuge at 12,000*g* for 1 minute.
6. Transfer the filtrate to a new 2 mL microcentrifuge tube (not provided). Add 5 µL RNase A. Let sit at room temperature for 5 minutes.
7. Add 525 µL RBB Buffer and 525 µL XP2 Binding Buffer. Vortex to mix thoroughly.
8. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
9. Transfer 750 µL lysate from Step 6 to the HiBind® DNA Mini Column. Centrifuge at 12,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.

BIND

10. Repeat Step 8 to transfer the remaining lysate.
11. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at 12,000*g* for 1 minute. Discard the filtrate and reuse collection tube.
12. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 12,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.

WASH

13. Repeat Step 12 for a second DNA Wash Buffer Step.
14. Centrifuge the empty HiBind® DNA Mini Column at 12,000*g* for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
15. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).
16. Add 50-100 µL Elution Buffer heated to 65°C directly to the center of the column membrane. Let sit at room temperature for 1 minute. Centrifuge at 12,000*g* for 1 minute.

ELUTE

17. Transfer the filtrate to the center of the HiBind® DNA Mini Column membrane. Let sit at room temperature for 1 minute. Centrifuge at 12,000*g* for 1 minute.
18. Store filtrate containing DNA at -20°C.

Disruption of Plant Tissue

- Grind samples with pestle

  A) Dry Specimens

  Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples, place ~15 mg dried tissue into a microcentrifuge tube (1.5 mL tubes are recommended) and grind using a pellet pestle. A fine powder will ensure optimal DNA extraction and yield.

  B) Fresh/Frozen Specimens

  Due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ~30 mg for first time users. It is very important to not overload the HiBind® DNA Mini Column. Too much starting material will decrease the yield and purity due to inefficient lysis. We recommend starting with 30 mg tissue. If results obtained are satisfactory, then increase amount of starting material. Best results are obtained with young leaves or needles.

  To prepare samples, collect tissue in a 1.5 mL or 2 mL microcentrifuge tube and dip the tube in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable Kontes pellet pestles. Alternatively, allow the liquid nitrogen to evaporate and store the samples at -70°C for later use. Transfer the ground sample into a 1.5 mL microcentrifuge tube.

  Note: Do not allow the sample to thaw during handling and weighing. To prevent the sample from thawing, keep the samples on a bed of dry ice.

- Disrupt Samples With Commercial Homogenizers

  Fresh, frozen, and dried plant tissue can be effectively disrupted and homogenized by rapid agitation in the presence of beads.

  1. Add two 3-4 mm stainless steel beads or ceramic beads to each vial.
  2. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample.
  3. Close the individual vial.
  4. Place the racks or plates into the clamps of the homogenizer.
  5. Homogenize for 60-90 seconds at 30 Hz. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads.
  6. Continue to Step 3 of the DNA Extraction and Purification Protocol on the reverse page.