E.Z.N.A.® Plant & Fungal DNA Kit

Revision No: 1.0

Quick Guide

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

Product	D3485-00	D3485-01	D3485-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
P1 Buffer	5 mL	50 mL	180 mL
P2 Buffer	1 mL	10 mL	40 mL
P3 Buffer	4 mL	20 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	60 mL
RNase A	40 µL	250 µL	1 mL

Supplied by user:

- Microcentrifuge capable of 14,000*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Incubator, heat block, or water bath capable of 65°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- Sterile deionized water
- Paper towels
- Grinding/homogenization equipment
- Optional: vacuum manifold

Before starting:

- Prepare DNA Wash Buffer according to the directions on the bottle.
- Set incubator, heat block, or water bath to 65°C.
- Heat sterile deionized water to 65°C.
- Heat Elution Buffer to 65°C.
- Prepare an ice bucket.

DNA Extraction and Purification from Dried Samples

This is the most robust method for the isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Dried tissue samples will need to be ground or homogenized before beginning this protocol. Please refer to Pages 8-9 of the full manual found online at https://omegabiotek.com for details.

LYSE

1. Transfer 10-50 mg dried powdered tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided). Process in sets of four to six tubes: grind, add P1 Buffer, and begin the 65°C incubation step before preparing another set.
2. Add 800 µL P1 Buffer. Vortex to mix thoroughly to disperse all clumps. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.
3. Add 180 µL P2 Buffer. Vortex to mix thoroughly.
4. **For fungal samples only:** Let sit on ice for 5 minutes.

INHIBITOR REMOVAL

5. Centrifuge at 10,000*g* for 10 minutes.
6. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

Optional: Centrifuge at 10,000*g* for 1-2 minutes to collect any debris that may have been transferred in Step 6.

7. Add 0.7 volumes 100% isopropanol. Vortex to precipitate DNA. Immediately centrifuge at 14,000*g* for 2 minutes to pellet DNA. Carefully aspirate or decant and discard the supernatant. Make sure not to dislodge the DNA pellet.
8. Invert the microcentrifuge tube on a paper towel for 1 minute to allow any residual liquid to drain. It is not necessary to dry the DNA pellet.

BIND

9. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet. A brief incubation at 65°C may be necessary to completely dissolve the DNA.
10. Add 4 µL RNase A. Vortex to mix thoroughly. No additional incubation is required for RNase treatment.
11. Add 150 µL P3 Buffer and 300 µL 100% ethanol. Vortex immediately to obtain a homogenous mixture. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times may break up the precipitates.

Optional: This the starting point of the optional Vacuum Protocol. If use of a vacuum is desired, please refer to the Vacuum Protocol found on Page 26 of the full manual found at https://omegabiotek.com. If not using a vacuum manifold, proceed to Step 12.

WASH

12. Insert a HiBind® DNA Mini Column into a 2 mL collection tube.
13. Transfer the entire sample, including any precipitates that may have formed, to the HiBind® DNA Mini Column. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and the 2 mL collection tube.
14. Transfer the HiBind® DNA Mini Column to a new 2 mL collection tube.
15. Add 750 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 10,000*g* for 1 minute. Discard filtrate and reuse the collection tube.
16. Repeat Step 15 for a second DNA Wash Buffer step.
17. Centrifuge the empty HiBind® DNA Mini Column at 10,000*g* for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
18. Transfer the HiBind® DNA Mini Column to a new 1.5 mL microcentrifuge tube.

ELUTE

19. Add 50-100 µL Elution Buffer heated to 65°C. Let sit at room temperature for 3-5 minutes. Centrifuge at 10,000*g* for 1 minute.
20. Repeat Step 19 for a second elution step.
21. Store eluted DNA at -20°C.

DNA Extraction and Purification from Fresh/Frozen Samples

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants and fungi, sample size should be limited to ≤200 mg. Best results are obtained with young leaves or needles. Tissue samples will need to be ground or homogenized before beginning this protocol. Please refer to Pages 8-9 of the full manual found online at https://omegabiotek.com for details.

LYSE

1. Grind 100 mg fresh/frozen tissue samples in liquid nitrogen. Process in sets of four to six tubes: grind, add P1 Buffer, and begin the 65°C incubation step before preparing another set.
2. Transfer ground tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).
3. Add 600 µL P1 Buffer. Vortex to mix thoroughly to disperse all clumps.
4. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.
5. Add 140 µL P2 Buffer. Vortex to mix thoroughly.
6. **For fungal samples only:** Let sit on ice for 5 minutes.
7. Proceed to Step 5 of the DNA EXTRACTION AND PURIFICATION FROM DRIED SAMPLES protocol on the reverse page.