E.Z.N.A.® SP Plant DNA Kit

Revision No: 2.0

Quick Guide

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

Product	D5511-00	D5511-01	D5511-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
Homogenizer Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
SP1 Buffer	5 mL	40 mL	150 mL
SP2 Buffer	1 mL	12 mL	50 mL
SP3 Buffer	2 mL	40 mL	100 mL
RNase A	30 µL	275 µL	1.1 mL
SPW Buffer	5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	60 mL

Supplied by user:

- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Incubator, heat block, or water bath capable of 65°C
- Vortexer
- 100% ethanol
- Ice bucket or cryorack for microcentrifuge tubes
- Pestles
- Liquid nitrogen if using fresh/frozen samples

Before starting:

- Prepare SPW Buffer and SP3 Buffer according to the directions on the bottles.
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C
- Prepare an ice bucket or cryorack

DNA Extraction and Purification from Dried Samples

LYSE

1. Transfer 10-30 mg dried powdered tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).
2. Add 600 µL SP1 Buffer and 5 µL RNase A. Vortex at maximum speed to mix thoroughly. **Do not mix SP1 Buffer and RNase A before use.**

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.
3. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.
4. Add 210 µL SP2 Buffer. Vortex to mix thoroughly. Let sit on ice for 5 minutes. Centrifuge at maximum speed (≥10,000 x *g*) for 10 minutes.
5. Insert a Homogenizer Mini Column into a 2 mL Collection Tube (provided). Carefully transfer the supernatant to the Homogenizer Mini Column. Do not disturb or transfer any of the insoluble pellet.
6. Immediately centrifuge at maximum speed for 2 minutes.

   Note: Longer centrifugation does not improve yield. The Homogenizer Mini Column will remove most remaining precipitates and cell debris, but a small amount may pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in Step 7.

BIND

7. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube(not provided). Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.
8. Add 1.5 volumes SP3 Buffer diluted with 100% ethanol (see bottle for instructions). 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 precipitate.

BIND

9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
10. Transfer 650 µL sample to the HiBind® DNA Mini Column. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
11. Repeat Step 10 until all of the sample has been transferred to the column.

WASH

12. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.
13. Add 650 µL SPW Buffer diluted with 100% ethanol (see bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
14. Repeat Step 13 for a second SPW Buffer wash step.
15. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

16. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube(not provided).
17. Add 50-100 µL Elution Buffer heated to 65°C. Let sit at room temperature for 3-5 minutes. Centrifuge at maximum speed for 1 minute.
18. Repeat Step 17 for a second elution step.
19. Store eluted DNA at -20°C.

DNA Extraction and Purification from Fresh/Frozen Samples

LYSE

1. Grind fresh/frozen tissue samples in liquid nitrogen.
2. Transfer 50 mg ground tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).
3. Add 400 µL SP1 Buffer and 5 µL RNase A. Vortex at maximum speed to mix thoroughly. **Do not mix SP1 Buffer and RNase A before use.**

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.
4. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.
5. Add 140 µL SP2 Buffer. Vortex to mix thoroughly. Let sit on ice for 5 minutes. Centrifuge at maximum speed (≥10,000 x *g*) for 10 minutes.
6. Proceed to Step 5 of the DNA EXTRACTION AND PURIFICATION FROM DRIED SAMPLES protocol on the reverse page.