omega BIO-TEK
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Product Manual

E.Z.N.A.® Plant DNA DS Kit

D2411-00	5 preps
D2411-01	50 preps
D2411-02	200 preps

Manual Date: June 2023
Revision Number: v4.3

For Research Use Only

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E.Z.N.A.® Plant DNA DS Kit

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Introduction

The E.Z.N.A.® Plant DNA DS Kit is designed for efficient recovery of genomic DNA up to 30 kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides, polyphenols, or those having a lower DNA content. Up to 50 mg wet tissue can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications.

This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the unique binding system to increase yields and provide high-quality DNA. The system eliminates the need for chloroform extractions traditionally associated with CTAB-based lysis methods. Samples are homogenized and lysed in a high salt buffer containing CTAB, binding conditions are adjusted, and DNA is purified using a HiBind® DNA Mini Column. Salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization applications.

New In this Edition

June 2023
- Troubleshooting guide has been updated to include protocol for removal of any residual RNA that may be present in purified DNA.

March 2022
- A new 200 prep kit has been added and is now available for purchase.

July 2019
- XP2 Buffer has been renamed XP2 Binding Buffer. This is a name change only. The formulation has not changed.

Kit Contents

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
User Manual	✓	✓	✓

* 10 mM Tris HCl pH 8.5

Storage and Stability

All of the E.Z.N.A.® Plant DNA DS Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A must be stored at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D2411-00	10 mL
D2411-01	100 mL
D2411-02	400 mL

2. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D2411-00	2 mL
D2411-01	10 mL
D2411-02	32 mL

Disruption of Plant Tissues

1. 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 of dried tissues into a microcentrifuge tube (1.5 mL tubes are recommended) and grind using a pellet pestle. Disposable Kontes pestles work well. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. 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. However, for some plant species, increasing the starting material can increase DNA yield. 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. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples. 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.

Disruption of Plant Tissues

1. 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 with or without lysis buffer.

   For Fresh, Frozen and Lyophilized/Dried Tissue without lysis buffer

   1. Add two 3-4 mm stainless steel beads or ceramic beads to each vial.
   2. Close the individual vial.
   3. Place the racks or plates into the clamps of the homogenizer.
   4. Homogenize for 60-90 seconds at 30 Hz. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads.
   5. Continue to Step 2 of the Protocol on page 7

   For Fresh, Frozen and Lyophilized/Dried Tissue with lysis buffer

   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 Protocol on Page 7

E.Z.N.A.® Plant DNA DS Kit - Fresh/Frozen/Dry Tissue Protocol

Materials and Equipment to be 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
- Sample Disruption Method (See Pages 5-6)

Before Starting:

- Prepare the DNA Wash Buffer and HBC Buffer according to the instructions on Page 4.
- Heat the Elution Buffer to 65°C.

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) according to Pages 5-6. For best results use a commercial homogenizer if available. If homogenizing in the presence of CSPL Buffer and Proteinase K Solution, skip to Step 3 after homogenization is complete.

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.

4. Centrifuge at 12,000*g* for 3 minutes.

5. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.

6. Transfer 550 µL cleared supernatant to the Homogenizer Mini Column.

7. Centrifuge at 12,000*g* for 1 minute.

8. Transfer the filtrate to a new 2 mL microcentrifuge tube (not provided).

9. Add 5 µL RNase A. Let sit at room temperature for 5 minutes.

10. Add 525 µL RBB Buffer and 525 µL XP2 Binding Buffer. Vortex to mix thoroughly.

11. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

12. Transfer 750 µL lysate from Step 9 to the HiBind® DNA Mini Column.

13. Centrifuge at 12,000*g* for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 to transfer the remaining lysate.

16. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

17. Centrifuge at 12,000*g* for 1 minute.

18. Discard the filtrate and reuse collection tube.

19. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Centrifuge at 12,000*g* for 1 minute.

21. Discard the filtrate and reuse the collection tube.

22. Repeat Steps 19-21 for a second DNA Wash Buffer step.

23. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at 12,000*g* to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

25. Add 50-100 µL Elution Buffer heated to 65°C directly to the center of the column membrane.

26. Let sit at room temperature for 1 minute.

27. Centrifuge at 12,000*g* for 1 minute.

28. Transfer the filtrate to the center of the HiBind® DNA Mini Column membrane.

29. Let sit at room temperature for 1 minute.

30. Centrifuge at 12,000*g* for 1 minute.

31. Store filtrate containing DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged column	Carryover of debris	Following transfer from Step 7, cell debris may have transferred.
	Sample too viscous	Do not exceed suggested amount of starting material.
Problem	**Cause**	**Solution**
Low DNA yield	Incomplete disruption of starting material	Completely homogenize the sample.
	Poor lysis of sample	Decrease amount of starting material or increase amount of CSPL Buffer and Proteinase K Solution.
	DNA remains bound to column	Increase elution volume to 200 µL and incubate on column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Dilute DNA Wash Buffer by adding appropriate volume of 100% ethanol prior to use.
	Difficulty transferring sample after lysis	If you are unable to transfer 550 µL after lysis with CSPL Buffer increase the buffer amount so that 550 µL can be successfully transferred.

Problem	Cause	Solution
Problems in downstream applications	Salt carryover	DNA Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash spin, ensure that the column is dried by centrifuging 2 minutes at maximum speed.
	RNA contamination	Double the RNase A volume and incubation time and incubate at 70°C instead of room temperature at Step 9 on Page 8.
		Remove RNA from eluted DNA sample by following the steps outlined below. More components may need to be purchased separately. • Add 5 µL RNase A to the eluted DNA and incubate for 5 minutes at room temperature. • Add one volume RBB Buffer and one volume XP2 Buffer to the eluted DNA. • Vortex briefly to mix. • Insert a HiBind DNA Mini Column into a 2 mL Collection tube. • Transfer the solution to the HiBind DNA Mini Column and follow Steps 13-31 to rebind, wash, and elute DNA from column.

Notices & Disclaimers

For European Union Use.

RBB Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or *in vitro* diagnostics.

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