E.Z.N.A.® SP Plant DNA Kit

D5511-00	5 preps
D5511-01	50 preps
D5511-02	200 preps

April 2013

E.Z.N.A.® SP Plant DNA Kit

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Manual Revision: April 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions, allowing proteins and other contaminants to be removed. Since phenol/chloroform extractions are not needed time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA obtained using the E.Z.N.A.® nucleic acid purification system can be used directly for a wide range of applications such as PCR, Southern blotting, and restriction enzyme digestion.

The E.Z.N.A.® SP Plant DNA Kit provides an easy and rapid method for the isolation of high quality total cellular DNA from plant species containing high levels of phenolic compounds and polysaccharides. Up to 100 mg wet tissue (or 30 mg dry tissue) can be processed in less than 45 minutes.

If you are using the E.Z.N.A.® SP Plant DNA Kit for the first time, please read this booklet thoroughly and become familiar with the procedures prior to performing the experiment. Dried or fresh/frozen plant tissue sample is disrupted and lysed in a specially formulated detergent. Proteins, polysaccharides, and cellular debris are precipitated. Binding conditions are adjusted with a buffer/ethanol solution and the sample is transferred to a HiBind® DNA Mini Column. Two rapid wash steps remove any trace contaminants (i.e. polysaccharides, cellular debris), and pure DNA is eluted in water or a low ionic strength buffer.

Protocol Selection:

- Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens.

Dry Specimens (Page 9)	Ideal for processing ≤30 mg powdered tissue samples. DNA yields will vary and depend on genome size, ploidy, and sample age. Yields based on a 30 mg sample will range any where from 5 to 50 µg DNA.
Fresh/Frozen Specimens (Page 13)	Ideal for processing ≤100 mg fresh or frozen tissue. A 100 mg sample will typically yield 3-30 µg of DNA.

New in this edition:

- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.

Determine the absorbance of an appropriate dilution (20- to 50- fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

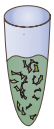
DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

If necessary the DNA can be concentrated. Add sodium chloride to reach a final concentration of 0.1M followed by 2X volumes 100% ethanol. Mix well and incubate at -20°C for 10 minutes. Centrifuge at 10,000 x *g* for 15 minutes and aspirate and discard the supernatant. Add 700 µL 70% ethanol and centrifuge at 10,000 x *g* for 2 minutes. Aspirate and discard the supernatant, air dry the pellet for 2 minutes, and resuspend the DNA in 20 µL sterile deionized water or 10 mM Tris-HCl, pH 8.5.

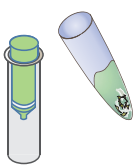
Illustrated Protocol

Grind the sample
in liquid nitrogen



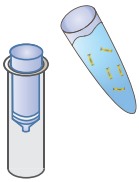
Lyse the sample
with SP1 Buffer

Precipitate proteins and
polysaccharides with SP2 Buffer



Transfer cleared debris to
a Homogenizer Column

Adjust binding
conditions



Transfer To a HiBind®
DNA Mini Column

Wash 2X

Dry

Elute

Kit Contents

Product Number	D5511-00	D5511-01	D5511-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
Homogenizer 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 Wash Buffer	5 mL	20 mL	3 x 20 mL
Elution Buffer	1.5 mL	15 mL	60 mL
Instruction Booklet	✓	✓	✓

**** SP3 Buffer contains a chaotropic salt, please take special precaution when handling this agent. SP3 Buffer is not compatible with disinfectants containing bleach.***

Storage and Stability

All of the E.Z.N.A.® SP Plant DNA Kit components are guaranteed for 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, a precipitate may form in SP1 Buffer and SP3 Buffer. Dissolve such deposits by warming each solution to 37°C with gentle shaking or stirring.

Preparing Reagents

- Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5511-00	20 mL
D5511-01	80 mL
D5511-02	80 mL per bottle

- Dilute SP3 Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5511-00	4 mL
D5511-01	80 mL
D5511-02	200 mL per bottle

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 and are available from Omega Bio-tek (Cat# SSI-1014-39 & SSI-1015-39). 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.

   Although various means of sample disruption can be used for this kit, such as beads or pestles, we recommend grinding the sample in liquid nitrogen. 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, which are available from OBI (Cat# SSI-1015-39). 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.

2. 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.

For Fresh, Frozen and Lyophilized/Dried Tissue

1. Add one 3-4 mm stainless steel bead to each well of a 96-well round-well plate.
2. Close the individual tubes with cap strips.
3. Freeze the sample in liquid nitrogen.

   Note: Lyophilized/Dried samples do not require freezing with liquid nitrogen.
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. Refer to manufacturer's protocol regarding use of liquid nitrogen with the homogenizer.

E.Z.N.A.® SP Plant DNA Mini Kit Protocol - Dried Samples

This is the most robust method for the isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping.

Materials and Equipment to be 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 (Recommended SSI-1014-39 or SSI-1015-39)

Before Starting:

- Prepare SPW Wash Buffer and SP3 Buffer according to the Preparing Reagents section on Page 6
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C
- Prepare an ice bucket or cryorack

Note: The recommended starting amount for dry tissue samples is 10-30 mg. Exceeding this amount will reduce yield and purity.

1. Prepare dry tissue samples. Refer to Pages 7-8 for details.

2. Transfer 10-30 mg dry powdered tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).

3. 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.

4. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by inverting the tube.

5. Add 210 µL SP2 Buffer. Vortex to mix thoroughly.

6. Let sit on ice for 5 minutes.

7. Centrifuge at maximum speed (≥10,000 x *g*) for 10 minutes.

8. Insert a Homogenizer Column into a 2 mL Collection Tube.

9. Carefully transfer the supernatant to the Homogenizer Column. Do not disturb or transfer any of the insoluble pellet.

10. Immediately centrifuge at maximum speed for 2 minutes.

    Note: Longer centrifugation does not improve yield. The Homogenizer 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 11.

11. Transfer cleared lysate to a 1.5 mL microcentrifuge tube (not supplied). Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

12. Add 1.5 volumes SP3 Buffer. 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.

    Note: SP3 Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

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

14. Transfer 650 µL sample to the HiBind® DNA Mini Column.

15. Centrifuge at maximum speed for 1 minute.

16. Discard filtrate and reuse the collection tube.

17. Repeat Steps 14-16 until all of the sample has been transferred to the column.

18. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

19. Add 650 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

20. Centrifuge at maximum speed for 1 minute.

21. Discard filtrate and reuse the collection tube.

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

23. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

24. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not supplied).

25. Add 50-100 µL Elution Buffer heated to 65°C.

26. Let sit at room temperature for 3-5 minutes.

27. Centrifuge at maximum speed for 1 minute.

28. Repeat Steps 25-27 for a second elution step.

    Note: Each elution step will typically yield of 60-70% of the DNA bound to the column. Thus two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. In some instances yields may be increased by incubating the column at 65°C (rather than at room temperature) upon the addition of Elution Buffer.

29. Store eluted DNA at -20°C.

E.Z.N.A.® SP Plant DNA Mini Kit Protocol - 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, sample size should be limited to ≤200 mg. Best results are obtained with young leaves or needles. This method isolates sufficient DNA for several tracks on a standard Southern assay.

Materials and Equipment to be 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
- Liquid nitrogen (for fresh/frozen samples)

Before Starting:

- Prepare SPW Wash Buffer and SP3 Buffer according to the Preparing Reagents section on Page 6
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C
- Prepare an ice bucket or cryorack

Note: Recommended starting amount for fresh/frozen samples is 50-100 mg. Exceeding this amount will reduce yield and purity

1. Grind fresh/frozen tissue samples in liquid nitrogen. Refer to Pages 7-8 for details.

2. Transfer 50 mg ground tissue to a nuclease-free 1.5 mL microcentrifuge tube (not provided).

   Note: Begin with 50 mg tissue. If results are satisfactory, increase the amount of starting material up to 100 mg.

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.

6. Let sit on ice for 5 minutes.

7. Centrifuge at maximum speed (≥10,000 x *g*) for 10 minutes.

8. Insert a Homogenizer Column into a 2 mL Collection Tube.

9. Carefully transfer the supernatant to the Homogenizer Column. Do not disturb or transfer any of the insoluble pellet.

10. Immediately centrifuge at maximum speed for 2 minutes.

    Note: Longer centrifugation does not improve yield. The Homogenizer 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 11.

11. Transfer cleared lysate to a 1.5 mL microcentrifuge tube (not supplied). Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

12. Add 1.5 volumes SP3 Buffer. 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.

    Note: SP3 Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

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

14. Transfer 650 µL sample to the HiBind® DNA Mini Column.

15. Centrifuge at maximum speed for 1 minute.

16. Discard filtrate and reuse the collection tube.

17. Repeat Steps 14-16 until all of the sample has been transferred to the column.

18. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

19. Add 650 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with ethanol before use. Please see Page 6 for instructions.

20. Centrifuge at maximum speed for 1 minute.

21. Discard filtrate and reuse the collection tube.

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

23. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

24. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not supplied).

25. Add 50-100 µL Elution Buffer heated to 65°C.

26. Let sit at room temperature for 3-5 minutes.

27. Centrifuge at maximum speed for 1 minute.

28. Repeat Steps 25-27 for a second elution step.

    Note: Each elution step will typically yield of 60-70% of the DNA bound to the column. Thus two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. In some instances yields may be increased by incubating the column at 65°C (rather than at room temperature) upon the addition of Elution Buffer.

29. Store eluted 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	Debris carryover	Following precipitation with SP2 Buffer, make sure no debris is transferred.
	DNA pellet not completely dissolved before transferring sample to column	Ensure that DNA is dissolved in water before adding SP3 Buffer and ethanol. May need to repeat incubation at 65°C.
	Sample too viscous	Do not exceed suggested amount of starting material. Alternatively, increase amounts of SP1 and SP2 Buffers and use two or more columns per sample.
	Incomplete precipitation with SP2 Buffer	Increase RCF or time of centrifugation after addition of SP2 Buffer.
Problem	Cause	Solution
Low DNA Yield	Incomplete homogenization	Completely homogenize sample.
	Prime columns	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
	Poor elution	Repeat Elution with increased elution volume. Incubate columns at 65°C for 5 minutes with Elution buffer
	Improper washing	SPW Wash Buffer must be diluted with 100% ethanol before use. If refrigerated, SPW Wash Buffer must be brought to room temperature.
	Overgrown culture	Overgrown culture contains lysed cells and degraded DNA.
Problem	Cause	Solution
Low A_{260}/A_{280} Ratio	Salt carryover	SPW Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash step, ensure that the column is dried by centrifuging 2 minutes at maximum speed.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
HiBind® DNA Mini Columns (200 columns)	DNACOL-02
1.5 mL DNase/RNase-free Microcentrifuge Tubes	SS1-1210-00
SP1 Buffer (250 mL)	PD086
SP2 Buffer (60 mL)	PD073
SP3 Buffer (100 mL)	PD074
Elution Buffer (100 mL)	PDR048
SPW Wash Buffer (25 mL)	PDR045
Homogenizer Columns (200 columns)	HCR003
RNase A (400 µL)	AC117

Notes:

Notes: