Contents

Introduction

E.Z.N.A.™ SP Fungal Kits are specially designed for rapid and reliable isolation of high-quality total cellular DNA from fungal species that contain high levels of phenolic compounds and polysaccharides. Up to 100 mg of wet tissue (or 25 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Omega Bio-Tek's HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. The newly introduced homogenization columns provide a fast and easy tool for sample homogenization. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the E.Z.N.A.™ SP Fungal Kit for the first time, please read this booklet to become familiar with the procedures. Dry or fresh fungal tissue is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Binding conditions are then adjusted and the sample is applied to a HiBind® DNA spin-column. Two rapid wash steps remove trace contaminants such as residual polysaccharides; then pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.™ SP Fungal Kits are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer SFG3. It is possible to dissolve such deposits by warming the solution at 37°C and gently shaking the container.

Kit Contents

Product Number	D5542-00	D5542-01	D5542-02
HiBind® DNA Columns	5	50	200
2 mL Collection Tubes	15	150	600
Omega Homogenizer Column	5	50	200
Buffer SFG1	5 mL	35 mL	135 mL
Buffer SFG2	1 mL	12 mL	50 mL
Buffer SFG3	3 mL	25 mL	80 mL
Equilibration Buffer	1.5 mL	7 mL	25 mL
SPW Buffer	5 mL	20 mL	3 x 20 mL
RNase A	25 µL	220 µL	850 µL
Elution Buffer	1.2 mL	15 mL	60 mL
Instruction Booklet	1	1	1

*Equilibration Buffer Contains Sodium Hydroixide. Always wear gloves and safety glasses when using.

Before Starting

Please read the entire booklet to become familiar with the E.Z.N.A.™ SP Fungal Miniprep Kit procedure.

- Equilibrate Elution Buffer at 65° C.
- Prepare **SFG3/ethanol** stock solution as follows

D5542-00	Add 6 mL absolute ethanol.
D5542-01	Add 50mL absolute ethanol per bottle.
D5542-02	Add 160 mL absolute ethanol per bottle.

- Dilute SPW Buffer concentrate with ethanol as follows and **store at room temperature**.

D5542-00	Add 20 mL absolute ethanol.
D5542-01	Add 80mL absolute ethanol.
D5542-02	Add 80 mL absolute ethanol per bottle.

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

A. Dry Specimens (Page 4)	For processing ≤25 mg powdered tissue. DNA yields vary depending on genome size, ploidy, and age of samples. Yields typically range from 5-50µg for 30 mg dry samples.
B. Fresh/Frozen Specimens (Page 6)	For processing ≤100 mg fresh (or frozen) tissue. Yields typically ranges from 3-30µg.

SP Fungal Miniprep Protocol

Dry Specimens

Materials to be provided by user

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Waterbath equilibrated to 65°C
- Absolute (96%-100%) ethanol

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

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 up to 25 mg of dried tissue into a microfuge tube 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 and 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 wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process in sets of four to six tubes until Step 2 before starting another set.

1. To 10-25 mg powdered dry tissue add 600 µL Buffer SFG1, followed by addition of 4 µL of RNase A solution. Mix throughly by votexing. Make sure to disperse all tissue clumps by pipetting or vortexing. Clumped tissue will not be lysed properly, resulting in lower DNA yields.
Note: The entire RNase A vial can be added directly to the bottle of Buffer SFG1. The RNase A will maintain its potency in Buffer SFG1 for several weeks.

2. Incubate at 65°C for 10-20 min. Mix sample 2 to 3 times during incubation by inverting the tube.

3. Add 210 µL Buffer SFG2 and vortex to mix. Incubate the samples for 5 minutes on ice. Centrifuge at ≥10,000 x g for 10 min.

4. Carefully aspirate supernatant to an Omega Homogenizer Column, making sure not to disturb the pellet or transfer any debris.

5. Immediately centrifuge at 10,000 x g for 2 min. Longer centrifugation does not improve yields. Omega Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 6.

6. Carefully transfer flow-through to a new 1.5 mL microtube (not supplied), making sure not to dislodge the pellet. Measure the volume of the lysate for next step.
Note: Using a set volume of lysate for each sample will eliminate the need for multiple lysate measurements.

7. Adjust binding conditions of the sample by adding 1.5 volumes of Buffer SFG3/ethanol mixture and vortex to obtain a homogeneous mixture. (E.g., 500 µl lysate would require 750 µl Buffer SFG3/ethanol mixture.)

8. Place a HiBind® DNA column into a 2 ml collection tube and add 100µl Equilibration Buffer. Let the column sit for 4 minutes. Spin at maximum speed for 20 seconds. Discard the flow-through liquid.

9. Transfer a maximum of 650 µL supernatant to a HiBind® DNA column inserted in a 2 mL collection tube (supplied). Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid and re-use the collection tube in Step 10.

10. Repeat Step 8 with remainder of sample, if any. Discard flow-through and collection tube.

11. Place the column into a new collection tube and add 650 µL SPW Buffer. Centrifuge at 10,000 x g for 1 min and discard the flow-through liquid. Reuse the collection tube in Step 12 below.
NOTE:SPW Buffer concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

12. Repeat wash step with an additional 650 µL SPW Buffer. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the 2 mL collection tube in Step 13.

13. Centrifuge empty column 2 min at maximum speed to dry the membrane. This step is critical for removal of residual ethanol that might otherwise be eluted with DNA and interfere with downstream applications.

14. Transfer column to a clean 1.5 mL tube (not supplied). Add 100 µL Elution Buffer, pre-warmed to 65ºC, and incubate at room temperature for 3 to 5 min. Centrifuge at 10,000 x g for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields.

15. **Repeat Step 13 with an additional 100 µL Elution Buffer.** This may be performed using another 1.5 mL tube to maintain a higher DNA concentration

in the first eluate. Alternatively, first eluate may be used for second elution to increase yield and concentration. Use of more than 200 µL Elution Buffer is not recommended.
TIP: To increase DNA concentration add buffer and incubate the column at 65° C for 5 min before elution.

Total DNA yields vary depending on type and quantity of sample. Typically, 5-50 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 25 mg dried tissue.

Fresh/Frozen Specimens

Materials to be provided by user
- Microcentrifuge capable of 10,000 x g
- Nuclease-free microfuge tubes
- Water bath equilibrated to 65ºC
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples

NOTE: Use extreme caution when handling liquid nitrogen.

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 fungal species, sample size should be limited to ≤100 mg. Best results are obtained with young leaves or needles. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in a 1.5 mL or 2 mL microfuge tube and freeze by dipping 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, one can allow liquid nitrogen to evaporate and then store samples at -70ºC 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.

1. Collect ground fungal tissue in a microfuge tube and immediately add 400 µL Buffer SFG1, followed by addition of 4 µL RNase A solution. Make sure to disperse all tissue clumps by pipetting or vortexing. Clumped tissue will not be lysed properly, resulting in lower DNA yields.
Note: The entire RNase A vial can be added directly to the bottle of Buffer SFG1. The RNase A will maintain its potency in Buffer SFG1 for several weeks.

2. Incubate at 65ºC for 10 min. Mix sample 2 to 3 times during incubation by inverting tube.

3. Add 140 µL Buffer SFG2 and vortex to mix. Centrifuge at ≥10,000 x g for 10 min.

4. Carefully aspirate supernatant to an Omega Homogenizer Column, making sure not to disturb the pellet or transfer any debris.

5. Immediately centrifuge at 10,000 x g for 2 min. Longer centrifugation does not improve yields. Omega Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 6.

6. Carefully transfer flow-through to a new 1.5 mL microtube (not supplied), making sure not to dislodge the pellet. Measure the volume of the lysate for next step.
   Note: Using a set volume of lysate for each sample will eliminate the need for multiple lysate measurements.

7. Adjust binding conditions of the sample by adding 1.5 volumes of Buffer SFG3/ethanol mixture and vortex to obtain a homogeneous mixture. (E.g., 500 µl lysate would require 750 µl Buffer SFG3/ethanol mixture.)

8. Place a HiBind® DNA column into a 2 ml collection tube and add 100µl Equilibration Buffer. Let the column sit for 4 minutes. Spin at maximum speed for 20 seconds. Discard the flow-through liquid.

9. Transfer a maximum of 650 µL supernatant to a HiBind® DNA column placed in a 2 mL collection tube (supplied). Centrifuge the column at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid and re-use the collection tube in Step 10.

10. Repeat Step 9 with remainder of sample, if any. Discard flow-through and collection tube.

11. Place the column into a new collection tube and add 650 µL SPW Buffer. Centrifuge at 10,000 x g for 1 min and discard the flow-through liquid. Reuse the collection tube in Step 12.
    NOTE:SPW Concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label

12. Repeat wash step with an additional 650 µL SPW Buffer. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse 2 mL collection tube in Step 13.

13. Centrifuge empty column 2 min at maximum speed to dry the membrane. This step is critical for removing residual ethanol that might otherwise be eluted with DNA and interfere with downstream applications.

14. Transfer column to a clean 1.5 mL tube (not supplied). Add 100 µL Elution Buffer, pre-warmed to 65°C, and incubate at room temperature for 3 to 5 min. Centrifuge at 10,000 x g for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields.

15. **Repeat Step 14 with an additional 100 µL of Elution buffer.** This may be performed using another 1.5 mL tube to maintain a higher DNA concentration in the first eluate. Alternatively, first eluate may be used for second elution to increase yield and concentration. Use of more than 200 µL Elution Buffer is not recommended.
    TIP: To increase DNA concentration add buffer and incubate the column at 65°C for 5 min before elution.

Total DNA yields vary depending on type and quantity of sample. Typically, 3-30 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 100 mg fresh leaf tissue.

Troubleshooting

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer SFG1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers SFG1 and SFG2.
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute SPW Buffer concentrate by adding appropriate volume of absolute ethanol prior to use (Page 3).
Problems in downstream applications	Salt carry-over.	SPW Buffer must be at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.