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Introduction

The E.Z.N.A.[®] SP Fungal DNA Midiprep Kit is specially designed for rapid and reliable isolation of high-quality total cellular DNA from Fungal species containing high levels of phenolic compounds and polysaccharides. Up to 500 mg of wet tissue (or 125 mg dry 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 Fungal tissue lysates. 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 DNA Midiprep 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. Binding conditions are then adjusted and the sample is applied to a HiBind[®] DNA midicolumn. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in water or low ionic strength 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 Buffers SFG1 and SFG3. It is possible to dissolve such deposits by warming the solutions at 37°C and gently shaking or stirring.

Kit Contents

Product Number	D5515-00	D5515-01	D5515-02
HiBind [®] DNA Midi Columns	2	10	25
15 ml Collection Tubes	2	10	25
Buffer SFG1	8 ml	40 ml	100 ml
Buffer SFG2	3 ml	12 ml	30 ml
Buffer SFG3	4 ml	20 ml	45 ml
SPW Buffer	5 ml	20 ml	2 x 20 ml
Elution Buffer	3 ml	12 ml	30 ml
RNase A Solution	32 µl	160 µl	400 µl
Midi Homogenizer Columns w/Tubes	2	10	25
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.[®] SP Fungal Midiprep Kit procedure.
- Equilibrate Elution Buffer and Buffer SFG1 at 65°C
- Prepare **SFG3/ethanol** stock solution as follows:

D5515-00 , Trial Kit	Add 8 ml absolute (96%-100%) ethanol.
D5515-01 , 10 preps	Add 40ml absolute (96%-100%) ethanol to each bottle.
D5515-02 , 25 preps	Add 90 ml absolute (96%-100%) ethanol to each bottle.
- Dilute SPW Buffer Concentrate with ethanol as follows and **store at room temperature**.

D5510-00 , Trial Kit	Add 20 ml absolute (96%-100%) ethanol.
D5515-01 , 10 preps	Add 80ml absolute (96%-100%) ethanol.
D5515-02 , 25 preps	Add 80 ml absolute (96%-100%) ethanol to each bottle.
- Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens. In addition, a short protocol is given for isolation of DNA for PCR reactions.

A. Dry Specimens (Page 4)	For processing up to 125 mg powdered tissue.
B. Fresh/Frozen Specimens (Page 6)	For processing up to 500 mg fresh (or frozen) tissue.

SP Fungal DNA Midiprep Protocol

A. Dry Specimens

Materials to be provided by user:

- Centrifuge capable of at least 8,000 x g
- Nuclease-free 15 ml or 20 ml high-speed centrifuge tubes (polycarbonate thick wall tubes preferred)
- Waterbath equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Ice or Cryorack for centrifuge tubes

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 125 mg of dried tissue into a 15 ml centrifuge tube (not supplied) and grind using a pellet pestle. 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 wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Mechanic grinder can also be used for grinding samples.

1. **To 30-125 mg powdered dry tissue add 3.5 ml Buffer SFG1 followed by addition of 14 µl RNase A stock solution. Mix thoroughly by vortexing. Make sure to disperse all clumps.**

Note: Make sure to disperse all tissue clumps by pipetting or vortexing. Clumped tissues will not be lysed properly and will result in lower DNA yields.

2. **Incubate at 65°C for 10 min.** Mix sample several times during incubation by inverting tube.
3. **Add 1 ml Buffer SFG2 and vortex to mix. Incubate the samples for 8 minutes on ice.**
4. **Centrifuge at 8,000 x g for 6 min at room temperature.**

Note: Some Fungal materials can generate very viscous lysates and large amounts of precipitates during this step. The preparation of a cleared lysate is essential to prevent clogging of the HiBind[®] DNA column. For some Fungal sample samples, it is difficult to make preparation of cleared lysate by a single centrifugation step because not all particulate matter forms a compact pellet. **Omega Homogenizer Columns in next step** can efficiently remove most cell debris and precipitates; therefore they make the preparation of a cleared lysate rapid and efficient.

5. **Carefully aspirate supernatant to an Omega Homogenizer Column, making sure not to disturb the pellet.**
6. **Centrifuge at 8,000 x g for 5 min.** Longer centrifugation does not improve yields. The Omega Homogenizer Column will remove most remaining precipitates and cell debris, but a small amount might pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 7.

7. **Carefully transfer cleared lysate into a new 15ml centrifuge tube; make sure not to dislodge the pellet.** Measure the volume of the lysate for next step.
8. **Adjust binding conditions of the sample by adding 1.5 volume of Buffer SFG3/ethanol mixture and immediately vortex to obtain a homogeneous mixture.**
9. **Transfer 3 ml of sample to a HiBind® DNA midicolumn placed in an 8 ml collection tube (supplied) .** Centrifuge the column at 8,000 x g for 5 min to bind DNA. Discard the flow-through liquid and re-use the collection tube for Step 10.
10. **Repeat Step 9 with remainder of sample, if any. Discard flow-through and reuse collection tube.**
11. **Place the column back into the collection tube and add 3.5 ml SPW Buffer.** Centrifuge at 8,000-10,000 x g for 3 min and discard the flow-through liquid. Reuse the collection tube in Step 12 below.

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 3.5 ml SPW Buffer.** Centrifuge at 8,000-10,000 x g for 3 min. Discard flow-through; reuse collection tube in Step 12.
13. **Centrifuge empty column (inserted into collection tube) 8 min at 8,000-10,000 x g to dry.** This step is *critical* for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications
14. **Transfer column to a clean 15 ml tube. Apply 400-500 µl Elution Buffer pre-warmed to 65°C and incubate at room temperature for 5 min.** Centrifuge at 8,000 x g for 3 min to elute DNA. Smaller volumes will significantly increase DNA concentration but result in lower yields.
15. **Repeat Step 14 with an additional 400-500 µl of buffer.** This step may be performed using another 15 ml tube to maintain a higher DNA concentration in the first eluate.

TIP: To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution. Alternatively, DNA concentration can be increased by using first eluate for a second elution.

Total DNA yields vary depending on type and quantity of sample.

B. Fresh/Frozen Specimens

Materials to be provided by user:

- Centrifuge capable of at least 8,000 x g
- Nuclease-free 15 ml or 20 ml high-speed centrifuge tubes (polycarbonate thick wall tubes preferred)
- Water bath equilibrated to 65°C
- Equilibrate Elution Buffer at 65°C.
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples
- Ice or Cryorack for microcentrifuge tubes

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 Fungals, sample size should be limited to ≤500 mg. Best results are obtained with young leaves or needles.

To prepare samples collect tissue in a 30 ml mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using a clean pestle. Alternatively, one can allow liquid nitrogen to evaporate and then store 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.

1. **Collect ground Fungal tissue (up to 500 mg) in a 15 ml centrifuge tube (not supplied) and add 2.5 ml Buffer SFG1 followed by addition of 14 µl Rnase A stock solution.** Mix thoroughly by vortexing. Make sure to disperse all clumps. .

Note: Make sure to disperse all tissue clumps by pipetting or vortexing. Clumped tissues will not be lysed properly and will result in lower DNA yields.

2. **Incubate at 65°C for 10 min. Mix sample several times during incubation by inverting tube.**
3. **Add 700 µl Buffer SFG2 and vortex to mix. Incubate the samples for 8 minutes on ice.**
4. **Centrifuge at 8,000 x g for 6 min at room temperature.**

Note: Some Fungal materials can generate very viscous lysates and large amounts of precipitates during this step. The preparation of a cleared lysate is essential to prevent clogging of the HiBind® DNA column. For some Fungal sample samples, it is difficult to make preparation of cleared lysate by a single centrifugation step because not all particulate matter forms a compact pellet. **Omega Homogenizer Columns** can efficiently remove most cell debris and precipitates; therefore they make the preparation of a cleared lysate rapid and efficient.

5. **Carefully aspirate supernatant to an Omega Homogenizer Column,**

making sure not to disturb the pellet.

6. **Centrifuge at 8,000 x g for 5 min.** Longer centrifugation does not improve yields. The Omega Homogenizer Column will remove most of the remaining precipitates and cell debris, but a small amount might pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 6.
7. **Carefully transfer flow-through into a new 15 ml microtube; make sure not to dislodge the pellet.** Measure the volume of the lysate for next step.
8. **Adjust binding conditions of the sample by adding 1.5 volume of Buffer SFG3/ethanol mixture and immediately vortex to obtain a homogeneous mixture.**
9. **Transfer 3 ml of sample to a HiBind® DNA column placed in an 8 ml collection tube (supplied) .** Centrifuge the column at 8,000 x g for 5 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 reuse collection tube.**
11. **Place the column back into the collection tube and add 3.5 ml SPW Buffer.** Centrifuge at 8,000-10,000 x g for 3 min and discard the flow-through liquid. Reuse the collection tube in Step 12 below.

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 3.5 ml SPW Buffer.** Centrifuge at 8,000-10,000 x g for 3 min. Discard flow-through; reuse collection tube in Step 13.
13. **Centrifuge empty column (inserted into collection tube) 8 min at 8,000-10,000 x g to dry.** This step is **critical** for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
14. **Transfer column to a clean 15 ml tube. Apply 400-500 µl Elution Buffer pre-warmed to 65°C and incubate at room temperature for 5 min. Centrifuge at 8,000 x g for 3 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but result in lower yields.
15. **Repeat Step 14 with an additional 400-500 µl of Elution buffer.** This step may be performed using another 15 ml tube to maintain a higher DNA concentration in the first eluate.

TIP: To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution. Alternatively, DNA concentration can be increased by using first eluate for a second elution.

Troubleshooting

Problem	Cause	Suggestions
Clogged column	Carry-over of debris.	Following precipitation with Buffer SFG2, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column.	In Protocols A and B, ensure that DNA is dissolved in water before adding Buffer SFG3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers SFG1 and SFG2.
	Incomplete precipitation following addition of P2.	Increase RCF or time of centrifugation after addition of buffer SFG2.
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 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 8 min at maximum speed.