Contents

Revised August 2006

Introduction

E.Z.N.A.™ Fungal DNA Midi Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of Fungal species and tissues. Up to 1g of wet tissue (or 150 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of 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.™ Fungal DNA Midi Kit for the first time, please read this booklet to become familiar with the procedures before beginning. 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, 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.™ Fungal DNA Midi Kit 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 FG3. It is possible to dissolve such deposits by warming the solution at 37°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product Number	D3590-01	D3590-02
HiBind® DNA Midi Columns	10	25
15 mL Collection Tubes	10	25
Buffer FG 1	50 mL	120 mL
Buffer FG 2	12 mL	30mL
Buffer FG 3	30 mL	80 mL
DNA Wash Buffer Concentrate	20 mL	2 x 20 mL
Elution Buffer	15 mL	30 mL
Instruction Booklet	1	1

Before Starting

- Prepare an RNase A stock solution at 20 mg/mL and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 20 µl of this solution.
- **Prepare the FG3/ethanol mixture by mixing 0.5 volume of FG3 and 1 volume of ethanol.**
- **Dilute DNA Wash Buffer Concentrate** with ethanol as follows and **store at room temperature**.

D3590-01	Add 80 mL absolute (96%-100%) ethanol
D3590-02	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 in 60 minutes.

A. Dry Specimens(page 4)	For processing ~200 mg powdered tissue.
B. Fresh/Frozen Specimens (page 6)	For processing ≤1 g fresh (or frozen) tissue.
C. Short protocol (page 9)	Rapid protocol for dried or fresh samples.

Fungal Midi DNA Kit Protocol

Materials to be provided by user:

- Centrifuge capable of at least 8,000 x g
- Nuclease-free 15 mL or 20 mL high speed Carbonate Thick wall centrifuge tubes
- Waterbath equilibrated to 65°C
- Equilibrate sterile dH_2O water or DNA Elution Buffer at 65°C.
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/mL

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 ~200 mg of dried tissue and grind using a mortar and pestle. 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 50-150 mg powdered dry tissue add 4 mL Buffer FG1 and 20 µl RNase A (20mg/mL).** Vortex vigorously to mix. Make sure to disperse all clumps.
2. **Incubate at 65°C for 30-60 min. Mix sample twice during incubation by inverting tube.**
3. **Add 1 mL Buffer FG2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at ≥8,000 x g for 20 min.**
4. **Carefully Transfer the supernatant and to a new 15 mL tube making sure not to loosen the pellet. Measure the volume of the sample and add 1.5 volume of prepared FG3/ethanol mixture** (see instruction in **Before Starting**). Vortex the sample to mix throughly.
5. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA Midi column placed in a 15 mL collection tube (supplied) .** Centrifuge the column at 8,000 x g for 5 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.
6. **Place column to a same collection tube and wash by adding 3.5 mL DNA Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 8,000 x g for 5 min and discard the flow-through liquid. Reuse the collection tube in Step 7 below.

   NOTE: DNA Wash Buffer Concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

7. **Repeat wash step with an additional 3.5 mL DNA Wash Buffer.** Centrifuge at 8,000 x g for 5 min. Discard flow-through and reuse 15 mL collection tube in step 8.

8. **Centrifuge empty column 15 min at 8000 x g to dry the columns.** This step is *critical* for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

9. **Transfer column to another clean 15 mL collection tube (not supplied with this kit). Apply 500 µl Elution Buffer (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 3 min. Centrifuge at 8,000 x g for 10 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 2 mL of buffer for elution is *not* recommended.

10. **Repeat Step 9 with an additional 500 µl of Elution Buffer.** This may be performed using another 15 mL collection tube (not supplied) 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 10 min before elution.

Total DNA yields vary depending on type and quantity of sample. Typically, 50-250 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 250 mg dried tissue.

B. Fresh/Frozen Specimens

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 in fungi, sample size should be limited to ≤1 g.
To prepare samples collect tissue in a 30mL mortar and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using clean pestles. 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 surface clean between samples.

1. **Collect ground Fungal tissue (start with 500 mg) in a 15 mL tube and immediately add 3mL Buffer FG 1 and 20 µL RNase A (20mg/mL).** Vortex vigorously to mix the sample. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

2. **Incubate at 65°C for 30-60 min. Mix sample twice during incubation by inverting tube.**

3. **Add 700 µl Buffer FG 2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at ≥8,000 x g for 10 min.**

4. **Carefully transfer supernatant into a new 15 mL centrifuge tube making sure not to loosen the pellet. Measure the volume of the sample and add 1.5 volume of prepared FG3/ethanol mixture** (see instruction in **Before Starting**). Vortex the sample to mix throughly.

5. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® DNA Midi-column placed in a 15 mL collection tube (supplied) .** Centrifuge the column at 8,000 x g for 5 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.

6. Follow Step 6-10 of Dry Specimens on Page 4-5.

C. Optional protocol

This protocol is designed for isolation of genomic DNA from fresh, frozen, or dried specimens from fungal samples contains higher phenolic material and polysaccharides which could cause lower yield or clogging of column with standard protocol A and B.

Follow suggestions for preparation of dried or fresh samples as outlined in Sections A and B (pages 4 and 6, respectively). Note the following limitations on sample size:

- **Dry Samples - use a maximum of 150 mg ground tissue**
- **Fresh Samples - use a maximum of 500 mg fresh/frozen ground tissue**

1. **Collect ground sample in a centrifuge tube and add 4mL Buffer FG1 and 20 µl RNase (20 mg/mL)**. Vortex vigorously to mix

2. **Incubate at 65°C for at least 25 min.** Mix sample once during incubation by inverting tube.

3. **Add 750 µl Buffer FG2 and vortex to mix. Incubate on ice for 5 minutes. Centrifuge at 8,000-10,000 x g for 10 min.**

4. **Carefully transfer cleared lysate to a new high speed centrifuge tube making sure not to disturb the pellet or transfer any debris. Add 0.7 volume isopropanol and vortex to precipitate DNA.** This step will remove much of the polysaccharides and improve spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow. No incubation is required after addition of isopropanol.

   TIP: In most cases 2.5 mL supernatant can easily be removed. This will require 1.75mL (0.7 volume) isopropanol. Note that depending on the sample type, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

5. **Immediately centrifuge at 8,000 x g for 15 min to pellet DNA.** Longer centrifugation does not improve yields.

6. **Carefully aspirate or decant the supernatant and discard making sure not to**

loosen the DNA pellet. Invert the centrifuge tube and place on a paper towel for 5 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

7. **Add 1.5 mL of sterile deionized water pre-heated to 65°C and vortex to resuspend the pellet.** A brief incubation at 65°C may be necessary to effectively dissolve the DNA. Add 20 µL RNase (20 mg/mL) and mix. No additional incubation is required for RNase treatment.

   TIP: While incubating at 65°C to dissolve the DNA, label and place the required number of HiBind ® DNA columns in 15 mL collection tubes (supplied).

8. **Adjust binding conditions of the sample by adding 750 µl Buffer FG3 followed by 1.5 absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation. A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation. Break the precipitation by pipetting up and down 10-20 times to obtain a homogeneous mixture.

9. **Apply the entire sample (including any precipitate that may have formed) to a HiBind® Midi-spin DNA column placed in a 15 mL collection tube (supplied) .** Centrifuge the column at 8,000 x g for 10 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.

10. **Reassemble column to same collection tube and wash by adding 3.5 mL Wash Buffer diluted with absolute (96%-100%) ethanol.** Centrifuge at 8,000 x g for 10 min and discard the flow-through liquid. Reuse the collection tube in Step 11 below.

11. **Repeat wash step with an additional 3.5 mL Wash Buffer.** Centrifuge at 8,000 x g for 5 min. Discard flow-through and reuse 15 mL collection tube in Step 12.

12. **Centrifuge empty column 10 min at 8,000 x g to dry the column.** This step is ***critical*** for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

13. **Transfer column to a clean 15 mL tube (not supplied with this kit). Apply 500 µl DNA Elution Buffer (or sterile deionized water) pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 8,000 x g for 10 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 2mL of buffer for elution is *not* recommended.

14. **Repeat Step 13 with an additional 0.5 mL of Elution Buffer.** This 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.

15. Store the eluted DNA at -20°C

Trouble Shooting

Problem	Cause	Suggestions
Clogged column	Carry-over of debris.	Following precipitation with Buffer FG 2, make sure no particulate material is transferred.
	Sample is not fully homogenized or DNA pellet not completely dissolved before applying sample to column.	In protocols A and B, ensure that DNA is completely homogenized by vortexiing before load to the column. In protocols A and B, ensure that DNA is dissolved in water before adding Buffer FG 3 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 FG 1 and FG 2 and use two or more columns per sample.
	Incomplete precipitation following addition of FG 2.	Increase RCF or time of centrifugation after addition of buffer FG 2.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer FG 1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers FG 1 and FG 2.
	DNA remains bound to column.	Increase elution volume to 1mL and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over.	Wash 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