Contents

Introduction

The E.Z.N.A.® Fungal DNA Maxi Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of Fungal species and tissues. Up to 2 g of wet tissue (or 500 mg dry tissue) can be processed for each column. 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 Maxi 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. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to a HiBind® DNA Maxi 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 Maxi Kit, except RNase A are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Store RNase A at -20°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.

Binding Capacity

Each HiBind™ DNA Maxi Column can bind up to 1 mg of genomic DNA. Use More than 2.5g Fresh Fungal samples or 500mg Dry Fungal samples are not recommend.

Kit Contents

Product Number	D3690-01	D3690-02
Purification Times	5 Preps	20 Preps
HiBind® DNA Maxi Columns	5	20
50 ml Collection Tubes	5	20
Buffer FG1	90 ml	2 x 180 ml
Buffer FG2	15 ml	60 ml
Buffer FG3	20 ml	80 ml
RNase A	300 µl	1.2 ml
Buffer HB	55 ml	220 ml
Elution Buffer	25 ml	100 ml
DNA Wash Buffer	40 ml	3 x 50 ml
Instruction Booklet	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Fungal DNA Maxi Kit procedures.

- Dilute DNA Wash Buffer with ethanol as follows and **store at room temperature**.

D3690-01	Add 160 ml absolute (96%-100%) ethanol to each bottle.
D3690-02	Add 200 ml absolute (96%-100%) ethanol to each bottle

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

A. Dry Specimens (Page 4)	For processing up to ~500 mg powdered tissue.
B. Fresh/Frozen Specimens (Page 6)	For processing up to ~2.5 g fresh (or frozen) tissue.

E.Z.N.A.™ Fungal DNA Maxi Protocol For Dry Specimens

Materials to be provided by user:

- High speed centrifuge capable of at least 10,000 x g
- Centrifuge with swinging-bucket rotor at room temperature capable of 4000xg.
- Nuclease-free 50 ml high speed centrifuge tubes (such as Nelgen polycarbonate tube Cat#3118-0050) and 50ml centrifuge tubes capable of 4000 x g centrifugation.
- Waterbath equilibrated to 65°C
- Equilibrate Elution Buffer
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol

Drying allows storage of field specimens for a prolonged period 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 ~500 m g of dried tissue into a 50 ml centrifuge tube and grind using a mortar and 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. Process in sets of four to six tubes until Step 2 before starting another set.

1. **To up to 500 mg powdered dry tissue add 16 ml Buffer FG1.** Vortex vigorously to mix. Make sure to disperse all clumps.

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

3. **Add 2.8 ml Buffer FG2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at 10,000 x g for 15 min.**

4. **Carefully aspirate supernatant to a new 50 ml hi-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 polysaccharide content and improves spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow.

   TIP: In most cases 16 ml supernatant can easily be removed. This will require 11.2 ml isopropanol. Note that depending on the sample, the volume of supernatant may vary.

5. **Centrifuge at 10,000 x g for 15 min to pellet DNA.**

6. **Carefully aspirate or decant the supernatant and discard making sure not to dislodge the DNA pellet.** Invert the centrifuge tube on a paper towel for 5 min to allow residual liquid to drain. **Do not over dry the DNA pellet**.

7. **Add 4 ml of sterile deionized water pre-heated to 65°C and 50 µl RNase A (Supplied). Vortex to resuspend the pellet.** Incubate at 65°C for 10-20 minutes to dissolve the DNA Pellet. Incubation time depends on amount and type of tissue, but is usually under 10 minutes. Some samples may require long time incubation.

8. **Adjust binding conditions of the sample by adding 2 ml Buffer FG3 followed by 4 ml absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

   Note: Step 9 to 15 should be performed in swinging-bucket rotor for maximal DNA yields. And all centrifugation steps must be carried out at room temperature.

9. **Apply the entire sample (including any precipitate that may have formed) to an HiBind® DNA Maxi-column placed in a 50 ml collection tube (supplied) .** Centrifuge the column at 4,000 x g for 5 min to bind DNA. Discard both the 50 ml collection tube and the flow-through liquid.

10. **Transfer column to a second 50ml collection tube (not supplied) and wash by adding 10 ml HB Buffer.** Centrifuge at 4,000 x g for 5 min and discard the flow-through liquid. Reuse the collection tube in Step 11 below.

11. **Add 15 ml DNA Wash Buffer to the column.** Centrifuge at 4,000 x g for 5 min. Discard flow-through and reuse 50 ml collection tube in Step 12.

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

12. **Wash the column with 15 ml DNA Wash Buffer to the column by centrifuge at 4,000 x g for 3 min. Discard the floe through and reuse the 50 ml collection tube for set 13.**

13. **Centrifuge empty column 20 min at 4,000x g to dry.** This step is critical for removing residual ethanol that may interfere with downstream applications.

14. **Transfer column to a clean 50 ml tube. Apply 1-2 ml Elution Buffer or pre-warmed to 65°C and incubate at room temperature for 5 min. Centrifuge at 4,000 x g for 10 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 4ml buffer for elution for elution is not recommended.

15. **Repeat Step 14 with an additional 1-2 ml Elution buffer.** This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first eluate. To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution.

E.Z.N.A.™ Fungal DNA Maxi Protocol For Fresh/Frozen Specimens

Materials to be provided by user:

- Hi-speed centrifuge capable of at least 10,000 x g
- Centrifuge with swinging-bucket rotor at room temperature capable of 4000xg.
- Nuclease-free 50 ml high speed centrifuge tubes .
- Waterbath equilibrated to 65°C
- Equilibrate Elution Buffer to 65°C
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol

NOTE: Use extreme caution when handling liquid nitrogen.

If available, mechanic tissue grinder will provider better result 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 no more than 2 g. 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 mortar. Grind the tissue using pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. 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. If available, mechanic tissue grinder will also provide good result

1. **Collect ground Fungal tissue (up to 2.5 g) in a 50 ml centrifuge tube which is capable of 10,000 x g, and immediately add 14 ml Buffer FG1.** Vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.
   Note: For best results, begin with 1g tissue. Increase amount of starting material depending on results.

2. **Incubate at 65°C for 30 min. Mix sample by vortxing during incubation.**

3. **Add 2.8 ml Buffer FG2 and vortex to mix. Incubate on ice for 10 minutes. Centrifuge at ≥10,000 x g for 10 min.**

4. **Carefully aspirate cleared lysate to a new 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 polysaccharide content and improves spin-column performance by increasing DNA binding capacity (and hence yield) in the steps that follow.

   TIP: In most cases 16 ml supernatant can easily be removed. This will require11.2 ml isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

5. **Centrifuge at 10,000 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 dislodge the DNA pellet.** Invert the centrifuge tube on a paper towel for 5 min to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

7. **Add 6 ml of sterile deionized water pre-heated to 65°C and 50 µl RNase A (Supplied). Vortex to resuspend the pellet.** Incubate at 65°C for 10-20 minutes to dissolve the DNA Pellet. Incubation time depends on amount and type of tissue, but is usually under 10 minutes. Some samples may require long time incubation.

8. **Adjust binding conditions of the sample by adding 3 ml Buffer FG3 followed by 6 ml absolute ethanol and vortex to obtain a homogeneous mixture.** A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

   Note: Step 9 to 15 should be performed in swinging-bucket rotor for maximal DNA yields. And all centrifugation steps must be carried out at room temperature.

9. **Apply the entire sample (including any precipitate that may have formed) to HiBind® DNA Maxi column placed in a 50 ml collection tube (supplied).** Centrifuge the column at 4,000 x g for 5 min to bind DNA. Discard both the 50 ml collection tube and the flow-through liquid.

10. **Transfer column to a second collection tube and add 10 ml HB Buffer.** Centrifuge at 4000 x g for 5 min and discard the flow-through liquid. Reuse the collection tube in Step 11 below.

11. **Add 10 ml DNA Wash Buffer diluted with ethanol to the column.** Centrifuge at 4,000 x g for 5 min. Discard flow-through and reuse 50 ml collection tube in Step 12.
    NOTE:DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

12. **Wash the column with another 10 ml of DNA Wash Buffer by centrifugation at 4,000 x g for 5 min. Discard the flow through and reuse the 50 ml collection tube for step 13.**

13. **Centrifuge empty column at 4,000 x g for 20 min 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.

14. **Transfer column to a clean 50 ml tube. Apply 1-2 ml Elution Buffer pre-warmed to 65°C and incubate at room temperature for 5 min. Centrifuge at 4,000 x g for 10 min to elute DNA.** Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 4 ml buffer for elution is not recommended.

15. **Repeat Step 14 with an additional 1-2 ml of buffer.** This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first eluate. To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution.

Troubleshooting

Problem	Cause	Suggestions
Clogged column	Carry-over of debris.	Following precipitation with Buffer FG2, 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 FG3 and ethanol. This may need repeated incubation at 65°C and vortexing. Remove un-dissolved material by centrifugation.
	Sample too viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers FG1 and FG2 proportionally.
	Incomplete precipitation following addition of FG2.	Increase RCF or time of centrifugation after addition of buffer FG2.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer FG1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers FG1 and FG2.
	DNA remains bound to column.	Increase elution volume to3 ml 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 10 min at 4000 x g.