

Contents

Introduction	2
Overview	2
Storage and Stability	2
Kit Contents	3
Before Starting	3
E-Z 96® MagBeads® Fungal DNA Isolation Protocol	4
A. Dry Specimens	4
B. Fresh/ Frozen Specimens	6
Troubleshooting	8

Revised June 2004

Introduction

E-Z 96® MagBeads® Fungal DNA Kits allow rapid and reliable isolation of high-quality genomic DNA from a wide variety of fungal species and tissues. Up to ninety-six 50 mg samples of wet tissue (or 15 mg dry tissue) per microplate can be processed in less than 1 hour. The system combines Omega Bio-Tek's EaZy Nucleic Acid® buffer chemistry with the convenience of MagBeads® particles to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. This kit is designed for manual or fully automated high throughput preparation of genomic, chloroplast, and mitochondrial DNA. 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 96® MagBeads® Fungal DNA Kit for the first time, please read this booklet to become familiar with the procedures. Dry or fresh fungal sample is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. The lysate is transferred to a 96-well microplate, binding conditions are adjusted, and genomic DNA binds to the MagBeads® particles. One or two rapid wash steps remove trace contaminants such as residual polysaccharides. And finally 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 96® MagBeads® fungal DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. For long term use, store MagBeads® Particles Solution at 2°C-8°C. For short term use, MagBeads® Particles Solution can be stored at room temperature for several weeks. During shipment or storage in cool ambient conditions, precipitates may form in Buffer FG1. It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Contents

Product Number	M1040-00 (Trial Kit)	M1040-01	M1040-02
Purifications	1 x 96	4 x 96	24 x 96
MagBeads® Particles Solution B	1.05 ml	4.2 ml	24.5 ml
Buffer FG1	45 ml	180 ml	2 x 500 ml
Buffer FG2	18 ml	60 ml	350 ml
MGB Binding Buffer	5 ml	34 ml	4 x 60 ml
SPM Wash Buffer	7.5 ml	51 ml	4 x 75 ml
DNA Elution Buffer	15 ml	50 ml	2 x 125 ml
RNase A	150 µl	600 µl	2 x 1.8 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E-Z 96® MagBeads® Fungal DNA Kit procedures.

- Equilibrate DNA Elution Buffer at 65°C

- Dilute MGB Binding Buffer with absolute ethanol (96%-100%) as follows and **store at room temperature**:

M1040-00, Trial Kit Add 32.5 ml absolute ethanol (96%-100%) to each bottle of MGB Binding Buffer.

M1040-01 Add 136 ml absolute ethanol (96%-100%) to each bottle of MGB Binding Buffer.

M1040-02 Add 200 ml absolute ethanol (96%-100%) to each bottle of MGB Binding Buffer.

- Diluted SPM Wash Buffer with absolute ethanol (96%-100%) as follows and **store at room temperature**:

M1040-00, Trial Kit Add 17.5 ml absolute ethanol to each bottle of SPM Buffer.

M1040-01 Add 119 ml absolute ethanol to each bottle of SPM Buffer.

M1040-01/M1040-02 Add 175 ml absolute ethanol to each bottle of SPM Buffer

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

A. Dry Specimens For processing ≤15 mg powdered tissue. .

B. Fresh/Frozen Specimens For processing ≤50 mg fresh (or frozen) tissue.

E-Z 96® MagBeads® Fungal DNA Isolation Protocol

A. Dry Specimens

Materials to be provided by user:

- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well microplate
- Magnetic Separation Stand for 96-well plates
- Incubator equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Equipment for disrupting fungal tissue (MM300 Mixer Mill or Geno/Grinder 2000 and Tungsten carbide beads)
- 8- or 12-channel pipette
- U- or V-bottom 96-well microplate
- 1.2 ml microtube rack or deep well 96-well microplate

This is the most robust method for isolation of total cellular (include mitochondrial, and genomic) DNA. 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 5-15 mg of dried tissue into a deep well plate or 1.2 ml microtube rack in the presence of Buffer FG1 and grinding bead. **For 5-15 mg dry sample add 400µl FG1 and 1µl RNase A.**

Note: FG1 and RNase A can be mixed in appropriate proportions to make a master mix before starting the procedure.

- Process in the mixer mill machine by following manufacturer's instructions. Time and speed will need to be determined for each type of sample.
- Incubate at 65°C for 10 min following sample disruption. Mix sample twice during incubation by inverting tube or vortexing plate very briefly.
- Add 140 µl Buffer FG2 and vortex to mix. Incubate 5 minutes on ice.
- Centrifuge at 3,000-5,000 x g for 10 min to pellet cell debris.
- Carefully aspirate 200µl supernatant to a U- or V-bottom microplate, making sure not to disturb the pellet or transfer any debris.
- Add 10µl/well of MagBeads® Particles Solution and follow by addition of 190µl/well of MGB Binding Buffer. Pipet to mix well.

NOTE: The MagBeads® Particles will settle and bead together at the bottom of their container after several hours. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT)

Tip: MGB Binding Buffer and MagBeads® Particles Solution can be combined in appropriate proportions to make a master mix before starting the procedure. Add 200 µl per well of MagBeads® Particle Solution/MGB Binding Buffer master mix to each well of the 96-well microplate.

8. Incubate for 5 minutes at room temperature, mixing once by pipetting or briefly vortexing. Use fresh tips to avoid cross-contamination.
9. Place the plate onto the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of each well adjacent to the magnets. (Supernatant should be clear when migration is complete.)
10. Remove the plate from the Magnetic Separation stand, then wash the pelleted MagBeads® particles by adding 300µl Buffer SPM diluted with ethanol. Resuspend the particles in Buffer SPM by pipetting or briefly vortexing plate. Again place the plate on the magnet separation stand and remove the supernatant after MagBeads® particles have completely migrated to the walls of the plate.

NOTE: For better washing efficiency, MagBeads® particles should be fully resuspended. Resuspension can be performed by pipetting or by vortexing.

11. **OPTIONAL:** Remove the plate from the magnetic separation stand, then wash the pelleted MagBeads® particles again by adding 200µl SPM Buffer. Resuspend the particles by pipetting or briefly vortexing plate. Place the plate on the magnetic separation stand and aspirate as much SPM as possible.

NOTE: This second wash step is strongly recommended for sensitive downstream applications such as real-time PCR.

12. Dry the MagBeads® particles for 5-10 min at room temperature (20-25 °C).

NOTE: It is absolutely critical that the MagBeads® particles dry completely before beginning elution step. Otherwise residual ethanol will contaminate samples and interfere with downstream applications.

13. Resuspend the MagBeads® particles in 50µl-100µl (depending on desired concentration) DNA Elution Buffer pre-heated to 65°C.
14. Incubate at room temperature for 5 minutes.
15. Place the plate on the magnetic separation stand and allow magnetic

particles to completely migrate toward magnetic source. Then transfer the DNA eluate to a clean 96-well microplate (not supplied).

B. Fresh/Frozen Specimens

Materials to be provided by user:

- Microcentrifuge capable of at least 10,000 x g
- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well microplate
- Magnetic Separation Stand
- Incubator equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Equipment for disrupting fungal tissue (MM300 Mixer Mill or Geno/Grinder 2000 and Tungsten carbide beads)
- 8- or 12-channel pipette
- U- or V-bottom 96-well microplate
- 1.5 or 2.0 ml microcentrifuge tubes, sealed deep-well plate or capped microtube rack for sample disruption

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 ≤50 mg. Best results are obtained with young leaves or needles.

1. Collect fungal tissue in a microfuge tube or deepwell plate (not supplied). Grind the samples as discussed below.

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 pellet pestles, which are available from Omega Bio-Tek (Cat# SS1015-39). **Add 300µl FG1 Buffer immediately and mix by vortexing.** 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.

To prepare samples in 96-well plate format, place sample in a sealed 96-well deep-well plate or capped microtube rack in the presence of 300µl of Buffer FG1 and one or two grinding beads. Process in the MM300 Mixture Mill or Geno/Grinder Mixture Mill following the manufacturer's instructions.

Note: Processing time and speed of the Mixture Mill will need to be determined for each sample type. Use the minimum speed that produces an

even suspension to prevent excess shearing of the DNA.

TIP: Process in sets of four to six tubes (if used): fill all tubes with liquid nitrogen, grind, add Buffer FG11 and proceed to Step 2 before starting another set. As a starting point use up to 30 mg sample per tube; if yield and purity are satisfactory increase to 50 mg.

2. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube or vortexing plate very briefly.
3. Add 105 µl Buffer FG2 and vortex to mix. Incubate on ice for 5 minutes.
4. Centrifuge at 3,000-5,000 x g (for deep-well plate) or ≥10,000 x g (for microtubes) for 10 min to pellet cell debris.
5. Carefully aspirate 200µl cleared lysate to a U- or V-bottom 96-well microplate, making sure not to disturb the pellet or transfer any debris.
6. Add 10µl/well of MagBeads® Particle Solution and follow by addition of 190µl/well of MGB Binding Buffer. Pipet to mix well.

NOTE: The MagBeads® Particles will settle and bead together at the bottom of their container after several hours. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT)

Tip: MGB Binding Buffer and MagBeads® Particles Solution can be combined in appropriate proportions to make a master mix before starting the procedure. Add 190 µl per well of MagBeads® Particle Solution/MGB Binding Buffer master mix to each well of the 96-well microplate.

7. Incubate for 5 minutes at room temperature, mixing once by pipetting or vortexing briefly. Use fresh tips to avoid cross-contamination.
8. Place the plate onto the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of each well adjacent to the magnets. (Supernatant should be clear when migration is complete.)
9. Remove the plate from the magnetic separation stand, then wash the pelleted MagBeads® particles by adding 250µl Buffer SPM. Resuspend the particles in Buffer SPM by pipetting or briefly vortexing the plate. Place the plate on the magnetic separation stand and remove the supernatant after MagBeads® particles have completely migrated to the walls of the plate.
10. **OPTIONAL:** Remove the plate from the magnetic separation Stand, then wash the pelleted MagBeads® particles again by adding 250µl SPM Buffer. Resuspend the particles by briefly vortexing plate. Place the plate on the magnetic separation stand and aspirate as much SPM as possible.

NOTE: This second wash step is strongly recommended for sensitive downstream applications such as real-time PCR.

11. Dry the MagBeads® particles for 5-10 min at room temperature (20-25 °C).

NOTE: It is absolutely critical that the MagBeads® particles dry completely before beginning elution step. Otherwise residual ethanol will contaminate samples and interfere with downstream applications.

12. Remove the plate from the magnetic separation stand to resuspend the MagBeads® particles in 50µl-100µl (depending on desired concentration) DNA Elution Buffer pre-heated to 65°C.
13. Place the plate on the magnetic separation stand and allow magnetic particles to completely migrate toward magnetic source. Then transfer the DNA eluate to a clean 96-well microplate (not supplied).

DNA yields vary depending on type and quantity of sample. Typically, 2-10 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 50 mg fresh leaf tissue.

Troubleshooting

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples make sure grind tissue completely.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers FG1 and FG2.
	DNA remains bound to magnetic beads	Increase elution volume to 100 µl and incubate at 65°C for 5 min before separating eluate.
	DNA washed off.	Dilute SPM Buffer by adding appropriate volume of absolute ethanol prior to use (Page 3).
Problems in downstream applications	Salt carry-over.	SPM Buffer must be at room temperature.
	Ethanol carry-over	Dry the magnetic beads pellet completely before adding elution buffer.