OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Fungal DNA Mini Kit

D3390-00	5 preps
D3390-01	50 preps
D3390-02	200 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Fungal DNA Mini Kit

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Manual Revision: May 2013

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Introduction

E.Z.N.A.®Fungal DNA Mini Kit allows for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of fungal species and tissues. Up to 200 mg of wet tissue (or up to 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Omega's HiBind® matrix with the speed and versatility of spin columns to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. 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 Mini Kit for the first time, please read this booklet to become familiar with the procedures. Dry or fresh fungal tissue is disrupted and 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 an HiBind® DNA Mini 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.

New in this Edition:

- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3390-00	D3390-01	D3390-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
FG1 Buffer	5 mL	50 mL	180 mL
FG2 Buffer	1 mL	10 mL	40 mL
FG3 Buffer	2 mL	20 mL	80 mL
RNase A	30 µL	275 µL	1.1 mL
DNA Wash Buffer	2 mL	20 mL	3 x 25 mL
Elution Buffer	1.5 mL	15 mL	50 mL
User Manual	✓	✓	✓

Storage and Stability

All components of the E.Z.N.A.® Fungal Mini Kit are stable for at least 24 months from date of purchase when stored at room temperature. During shipment, or storage in cool ambient conditions, precipitates may form in FG3 Buffer. It is possible to dissolve such deposits by warming the solution at 37°C.

Preparing Reagents

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3390-00	8 mL
D3390-01	80 mL
D3390-02	100 mL per bottle

Protocol Selection Guide

Protocol	Page	Ideal Sample
Dried Specimens	5	For processing ≤50 mg powdered tissue. Yield is sufficient for several tracks on Southern assay.
Fresh or Frozen Specimens	9	For processing ≤200 mg fresh (or frozen) tissue. Yield is similar to that for dried specimens.
Short Protocol	13	Rapid protocol for dried or fresh samples. Yield is sufficient for PCR.

E.Z.N.A.® Fungal DNA Mini Kit Protocol - Dried Specimens

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 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 ~50 mg of dried tissue into a microcentrifuge tube (2 mL tubes are recommended for processing of >50 mg tissue) and grind using a pellet pestle. Disposable Kontes pestles work well and are available from Omega Bio-tek (Cat# SSI-1015-39). 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 cleaning. 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.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes (Cat# SSI-1210-00 or SSI-1310-00)
- Water bath capable of 65°C
- Pestles for grinding tissue (Cat# SSI-1015-39)
- Sterile deionized water
- Isopropanol
- 100% ethanol
- Optional: 3M NaOH

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Heat the sterile deionized water and Elution Buffer to 65°C
- Prepare an ice bucket

1. Prepare 10-50 mg powdered dry tissue in a 1.5 or 2 mL microcentrifuge tube.

2. Add 800 µL FG1 Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.

   Note: Process in sets of four to six tubes: grind, add FG1 Buffer, then proceed to Step 3 before starting another set. Do not exceed 50 mg dried tissue.

3. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube.

4. Add 180 µL FG2 Buffer. Vortex to mix thoroughly.

5. Let sit on ice for 5 minutes.

6. Centrifuge at 10,000 x *g* for 10 minutes.

7. Transfer supernatant to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.

8. Add 0.7 volumes isopropanol. Vortex to precipitate DNA.

   Note: In most cases 700 µL supernatant can easily be removed. This will require 490 µL 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. 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. No incubation is required after addition of isopropanol.

9. Immediately centrifuge at 10,000 x *g* for 2 minutes. Longer centrifugation does not improve yields.

10. Aspirate and discard the supernatant, making sure not to dislodge the DNA pellet.

11. Invert the microcentrifuge tube on a paper towel for 1 minute to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

12. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet.

    Note: A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

13. Add 4 µL RNase A. Vortex to mix thoroughly.

14. Add 150 µL FG3 Buffer and 300 µL 100% ethanol. Vortex to mix thoroughly.

    Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

15. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 60 seconds.
4. Discard the filtrate and reuse the Collection Tube.

16. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

17. Centrifuge at 10,000 x *g* for 1 minute.

18. Discard the filtrate and the Collection Tube.

19. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

20. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

21. Centrifuge at 10,000 x *g* for 1 minute.

22. Discard the filtrate and reuse the Collection Tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

25. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube.

26. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

27. Let sit for 3 to 5 minutes.

28. Centrifuge at 10,000 x *g* for 1 minute.

29. Repeat Steps 26-28 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

30. Store DNA at -20°C.

E.Z.N.A.® Fungal DNA Mini Kit Protocol - Fresh or Frozen Specimens

This protocol is suitable for most fresh or frozen tissue samples. However, due to the tremendous variation in water and polysaccharide content of various fungi, sample size should be limited to ≤200 mg. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in a 1.5 or 2 mL microcentrifuge 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 Omega Bio-tek (Cat# SSI-1015-39). 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.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes (Cat# SSI-1210-00 or SSI-1310-00)
- Water bath capable of 65°C
- Pestles for grinding tissue (Cat# SSI-1015-39)
- Sterile deionized water
- Isopropanol
- 100% ethanol
- Optional: 3M NaOH

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Heat the sterile deionized water and Elution Buffer to 65°C
- Prepare an ice bucket

1. Prepare 100 mg tissue in a 1.5 or 2 mL microcentrifuge tube.

2. Add 600 µL FG1 Buffer. Vortex vigorously to mix. Make sure to disperse all clumps.

   Note: Process in sets of four to six tubes: grind, add FG1 Buffer, then proceed to Step 3 before starting another set. Do not exceed 200 mg tissue.

3. Incubate at 65°C for 10 minutes. Mix sample twice during incubation by inverting tube.

4. Add 140 µL FG2 Buffer. Vortex to mix thoroughly.

5. Let sit on ice for 5 minutes.

6. Centrifuge at 10,000 x *g* for 10 minutes.

7. Transfer supernatant to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.

8. Add 0.7 volumes isopropanol. Vortex to precipitate DNA.

   Note: In most cases 600 µL supernatant can easily be removed. This will require 420 µL 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. 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. No incubation is required after addition of isopropanol.

9. Immediately centrifuge at 10,000 x *g* for 2 minutes. Longer centrifugation does not improve yields.

10. Aspirate and discard the supernatant, making sure not to dislodge the DNA pellet.

11. Invert the microcentrifuge tube on a paper towel for 1 minute to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

12. Add 300 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet.

    Note: A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

13. Add 4 µL RNase A. Vortex to mix thoroughly.

14. Add 150 µL FG3 Buffer and 300 µL 100% ethanol. Vortex to mix thoroughly.

    Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

15. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 60 seconds.
4. Discard the filtrate and reuse the Collection Tube.

16. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

17. Centrifuge at 10,000 x *g* for 1 minute.

18. Discard the filtrate and the Collection Tube.

19. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

20. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

21. Centrifuge at 10,000 x *g* for 1 minute.

22. Discard the filtrate and reuse the Collection Tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

25. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube.

26. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will significantly increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

27. Let sit for 3 to 5 minutes.

28. Centrifuge at 10,000 x *g* for 1 minute.

29. Repeat Steps 26-28 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

30. Store DNA at -20°C.

E.Z.N.A.® Fungal DNA Mini Kit Protocol - Short Protocol

This simplified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for use in PCR reactions. The procedure limits the amount of starting material, so that DNA yields will generally be lower than those obtained with the previous protocols. The short protocol is not recommended for Southern analysis or cloning work, as in most cases there will be insufficient material.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes (Cat# SSI-1310-00)
- Water bath capable of 65°C
- Isopropanol
- 100% ethanol
- Liquid nitrogen
- β-mercaptoethanol
- Optional: 3M NaOH
- Optional: Sterile deionized water

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Heat the sterile deionized water and Elution Buffer to 65°C
- For dried specimens: use a maximum of 10 mg ground tissue
- For fresh/frozen specimens: use a maximum of 40 mg ground tissue
- Prepare an ice bucket

1. Prepare tissue in a 2 mL microcentrifuge tube.

2. Add 600 µL FG1 Buffer and 5 µL RNase A. Vortex vigorously to mix. Make sure to disperse all clumps.

3. Let sit for 1 minute.

4. Add 10 µL β-mercaptoethanol. Vortex to mix thoroughly.

5. Incubate at 65°C for at least 5 minutes. Mix sample once during incubation by inverting tube.

6. Add 140 µL FG2 Buffer. Vortex to mix thoroughly.

7. Let sit on ice for 5 minutes.

8. Centrifuge at 10,000 x *g* for 10 minutes.

9. Transfer supernatant to a new microcentrifuge tube, making sure not to disturb the pellet or transfer any debris.

   Note: In most cases 600 µL supernatant can easily be removed. The volume of supernatant will vary, and is usually lower with dried samples.

10. Add 0.5 volumes FG3 Buffer and 1 volume 100% ethanol. Vortex to mix thoroughly.

    Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

11. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Centrifuge at maximum speed for 60 seconds.
4. Discard the filtrate and reuse the Collection Tube.

12. Transfer 800 µL sample from Step 10 (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 until all of the sample has been transferred to the HiBind® DNA Mini Column.

16. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

17. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 or the bottle label for instructions.

18. Centrifuge at 10,000 x *g* for 1 minute.

19. Discard the filtrate and reuse the Collection Tube.

20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

21. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

22. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube.

23. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will significantly increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

24. Let sit for 3 to 5 minutes.

25. Centrifuge at 10,000 x *g* for 1 minute.

26. Repeat Steps 23-25 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

27. Store DNA at -20°C.

E.Z.N.A.® Fungal DNA Mini Kit Protocol - Vacuum/Spin Protocol

Note: Please read through previous sections of this manual before using this protocol.

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (Cat# VAC-08)
- Microcentrifuge capable of at least 10,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes (Cat# SSI-1310-00)
- Water bath capable of 65°C
- Sterile deionized water
- Isopropanol
- 100% ethanol
- Optional: 3M NaOH

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions on Page 4
- Heat the sterile deionized water and Elution Buffer to 65°C
- For dried specimens: use a maximum of 10 mg ground tissue
- For fresh/frozen specimens: use a maximum of 40 mg ground tissue
- Prepare an ice bucket

1. Complete Steps 1-14 of the "Dried Specimens" protocol on Page 5, Steps 1-14 of the "Fresh or Frozen Specimens" protocol on Page 9, or Steps 1-10 of the "Short Protocol" on Page 13.

2. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes.
3. Apply the vacuum until all the buffer has passed through the membrane.
4. Turn off the vacuum source.

3. Transfer the sample to the HiBind® DNA Mini Column.

4. Apply the vacuum until all the sample has passed through the membrane.

5. Turn off the vacuum source.

6. Add 750 µL DNA Wash Buffer.

   Note: DNA Wash Buffer must be diluted with 100% ethanol. Please see the instructions on Page 4.

7. Apply the vacuum until all the buffer has passed through the membrane.

8. Repeat Steps 6-7 for a second DNA Wash Buffer wash step.

9. Continue to apply maximum vacuum for an additional 10 minutes to dry the membrane.

   Note: It is critical to dry the membrane. Residual ethanol may interfere with downstream applications.

10. Turn off the vacuum source.

11. Transfer the HiBind® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube.

12. Add 100 µL Elution Buffer (or sterile deionized water) heated to 65°C.

    Note: Smaller elution volumes will significantly increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

13. Let sit for 3 to 5 minutes.

14. Centrifuge at 10,000 x *g* for 1 minute.

15. Repeat Steps 12-14 for a second elution step.

    Note: Any combination of the following steps can be used to help increase DNA yield.

    - After adding the Elution Buffer, incubate the column for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Elution Buffer (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

16. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged column	Debris carryover	Following precipitation with FG2 Buffer, 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 FG3 Buffer and ethanol. This may need repeated incubation at 65°C with vortexing.
	Sample too viscous	In protocol C, do not exceed suggested amount of starting material. Alternatively, increase the amounts of FG1 and FG2 Buffers and use two or more columns per sample.
	Incomplete precipitation following addition of FG2 Buffer	Increase speed or time of centrifugation after addition of FG2 Buffer.
Low DNA yield	Incomplete disruption of starting material	For both dry and fresh samples, obtain a fine homogeneous powder before adding FG1 Buffer.
	Poor lysis of tissue	Decrease amount of starting material or increase amount of FG1 and FG2 Buffers.
	DNA remains bound to column	Increase elution volume to 200 µL and incubate column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Dilute DNA Wash Buffer by adding the appropriate volume of ethanol prior to use (Page 4).
Problems in downstream applications	Salt carryover	DNA Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash step, ensure that the column is dried by centrifuging 2 minutes at maximum speed.

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