E.Z.N.A.® SP Fungal DNA Mini Kit

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Manual Revision: June 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

E.Z.N.A.® SP Fungal Mini Kits are specially designed for rapid and reliable isolation of high-quality total cellular DNA from fungal species that contain high levels of phenolic compounds and polysaccharides. Up to 100 mg wet tissue (or 25 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Omega Bio-tek's HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. The newly introduced homogenization columns provide a fast and easy tool for sample homogenization. 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.

If using the E.Z.N.A.® SP Fungal 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. Binding conditions are adjusted and the sample is transferred to a HiBind® DNA Mini Column. Two rapid wash steps remove trace contaminants such as residual polysaccharides and pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- 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	D5542-00	D5542-01	D5542-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
Homogenizer Mini Column	5	50	200
SFG1 Buffer	5 mL	35 mL	135 mL
SFG2 Buffer	1 mL	12 mL	50 mL
SFG3 Buffer	3 mL	25 mL	80 mL
SPW Wash Buffer	5 mL	20 mL	3 x 20 mL
RNase A	25 µL	220 µL	850 µL
Elution Buffer	1.2 mL	15 mL	60 mL
User Manual	✓	✓	✓

Storage and Stability

All components of the E.Z.N.A.® SP Fungal Mini Kit are stable for at least 24 months from date of purchase when stored as follows. RNase A should be stored at 2-8°C. All remaining components should be stored at room temperature. During shipment, or storage in cool ambient conditions, precipitates may form in SFG3 Buffer. It is possible to dissolve such deposits by warming the solution at 37°C with gentle shaking.

Preparing Reagents

1. Dilute SFG3 Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5542-00	6 mL
D5542-01	50 mL
D5542-02	160 mL

2. Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5542-00	20 mL
D5542-01	80 mL
D5542-02	80 mL per bottle

Protocol Selection Guide

Protocol	Page	Ideal Sample
Dried Specimens	5	For processing ≤25 mg powdered tissue. DNA yields vary depending on genome size, ploidy, and sample age. Yields typically range from 5-50 µg per 30 mg dried tissue .
Fresh or Frozen Specimens	9	For processing ≤100 mg fresh (or frozen) tissue. Yields typically ranges from 3-30 µg.

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

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast and genomic) DNA from dried fungal samples. 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 ~25 mg of dried tissue into a microcentrifuge tube 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. Process in sets of four to six tubes until Step 2 before starting another set.

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
- Water bath, incubator, or heat block equilibrated to 65°C
- Ice bucket
- 100% ethanol

Before Starting:

- Prepare SPW Wash Buffer and SFG3 Buffer according to the Preparing Reagents section on Page 4
- Prepare an ice bucket
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C

1. Transfer 10-25 mg dry powdered tissue to a nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not provided).

2. Add 600 µL SFG1 Buffer and 4 µL RNase A. Vortex at maximum speed to mix thoroughly.

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

3. Incubate at 65°C for 10-20 minutes. Mix sample 2 to 3 times during incubation by inverting the tube.

4. Add 210 µL SFG2 Buffer. Vortex to mix thoroughly.

5. Let sit on ice for 5 minutes.

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

7. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.

8. Transfer supernatant to a Homogenizer Mini Column, making sure not to disturb the pellet or transfer any debris.

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

   Note: The Homogenizer Mini Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 10.

10. Carefully transfer the lysate to a new 1.5 mL microcentrifuge tube (not provided), making sure not to dislodge the pellet. Measure the volume of the lysate for next step.

    Note: Using a set volume of lysate for each sample will eliminate the need for multiple measurements.

11. Add 1.5 volumes SFG3 Buffer. For example, 500 µL lysate would require 750 µL SFG3 Buffer. Vortex to obtain a homogeneous mixture.

    Note: SFG3 Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 for instructions.

12. 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.

13. Transfer 650 µL sample from Step 11 to the HiBind® DNA Mini Column.

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

15. Discard the filtrate and reuse the collection tube.

16. Repeat Steps 13-15 until all the remaining sample has been transferred to the HiBind® DNA Mini Column.

17. Transfer the column into a new 2 mL Collection Tube.

18. Add 650 µL SPW Wash Buffer.

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

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

20. Discard the filtrate and reuse the Collection Tube.

21. Repeat Steps 18-20 for a second SPW Wash Buffer wash step.

22. 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.

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

24. 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.

25. Let sit for 3 to 5 minutes at room temperature.

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

27. Repeat Steps 24-26 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).

28. Store DNA at -20°C.

E.Z.N.A.® SP Fungal DNA Mini Kit - Fresh/Frozen Specimen Protocol

This protocol is suitable for most fresh or frozen tissue samples that allows for more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of fungal species, sample size should be limited to ≤100 mg. Best results are obtained with young tissue. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in a 1.5 mL 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 OBI (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
- Water bath, incubator, or heat block equilibrated to 65°C
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples

Before Starting:

- Prepare SPW Wash Buffer and SFG3 Buffer according to the Preparing Reagents section on Page 4
- Set an incubator, heat block, or water bath to 65°C
- Heat Elution Buffer to 65°C

1. Transfer ≤100 mg ground fungal tissue to a nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not provided).

2. Add 400 µL SFG1 Buffer and 4 µL RNase A. Vortex at maximum speed to mix thoroughly.

   Note: Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

3. Incubate at 65°C for 10 minutes. Mix sample 2 to 3 times during incubation by inverting the tube.

4. Add 140 µL SFG2 Buffer. Vortex to mix thoroughly.

5. Centrifuge at ≥10,000 x *g* for 10 minutes.

6. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.

7. Transfer supernatant to a Homogenizer Mini Column, making sure not to disturb the pellet or transfer any debris.

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

   Note: The Homogenizer Mini Column will remove most remaining precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not disturb this pellet in Step 9.

9. Carefully transfer the lysate to a new 1.5 mL microcentrifuge tube (not provided), making sure not to dislodge the pellet. Measure the volume of the lysate for next step.

   Note: Using a set volume of lysate for each sample will eliminate the need for multiple measurements.

10. Add 1.5 volumes SFG3 Buffer. For example, 500 µL lysate would require 750 µL SFG3 Buffer. Vortex to obtain a homogeneous mixture.

    Note: SFG3 Buffer must be diluted with 100% ethanol prior to use. Please refer to Page 4 for instructions.

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 650 µL sample from Step 10 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 the remaining sample has been transferred to the HiBind® DNA Mini Column.

16. Transfer the column into a new 2 mL Collection Tube.

17. Add 650 µL SPW Wash Buffer.

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

24. Let sit for 3 to 5 minutes at room temperature.

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.

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
Low DNA yield	Incomplete disruption of starting material	For both dry and fresh samples, obtain a fine homogeneous powder before adding SFG1 Buffer.
	Poor lysis of tissue	Decrease amount of starting material or increase the amount of SFG1 Buffer and SFG2 Buffer.
	DNA remains bound to column	Increase elution volume to 200 µL and incubate the column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Dilute SPW Wash Buffer by adding the appropriate volume of 100% ethanol prior to use (see Page 4 for instructions).
Problem	Cause	Solution
Problems in downstream applications	Salt carryover	SPW Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash spin, ensure that the column is dried by centrifuging 2 minutes at maximum speed.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Homogenizer Mini Column, 50/200	HCR001/HCR003
Homogenization Pestles, 1.5 mL, 10/bag, 20 bags/cs	SSI-1015-39
SPW Wash Buffer, 25 mL	PDR045
RNase A, 5 mL	AC118
Elution Buffer, 100 mL	PDR048

Notes: