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We use this protocol and it's working

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FastDNA SPIN Kit for Soil

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ABSTRACT

The FastDNATM SPIN Kit for Soil quickly and efficiently isolates PCR-ready genomic DNA directly from soil samples in less than 30 minutes. Designed for use with the FastPrep® instruments from MP Biomedicals, plant and animal tissues, bacteria, algae, fungal spores and other members of a soil population are easily lysed within 40 seconds. These benchtop devices use a unique, optimized motion to homogenize samples by multidirectional, simultaneous impaction with lysing matrix particles. FastPrep instruments provide a quick, efficient and highly reproducible homogenization that surpasses traditional extraction methods using enzymatic digestion, sonication, blending, douncing and vortexing.

Samples are placed into 2.0 mL tubes containing Lysing Matrix E, a mixture of ceramic and silica particles designed to efficiently lyse all soil organisms, including historically difficult sources, such as eubacterial spores and endospores, gram positive bacteria, yeast, algae, nematodes and fungi. Homogenization in a FastPrep instrument with Lysing Matrix E takes place in the presence of MT Buffer and Sodium Phosphate Buffer, reagents carefully developed to protect and solubilize nucleic acids and proteins upon cell lysis. These reagents work synergistically to allow extraction of genomic DNA with minimal RNA contamination.

Following lysis, samples are centrifuged to pellet soil, cell debris and lysing matrix. DNA is purified from the supernatant with the Binding Matrix FastDNA procedure using SPIN filters. Eluted DNA is ready for PCR, restriction digest, electrophoresis and any other desired application.

GUIDELINES

The fill volume of the lysing matrix tube after addition of Sodium Phosphate and MT Buffers to the sample should allow sufficient air space in the sample tube for efficient FastPrep instrument processing. MP Bio recommends using up to 500 mg of most soil types. Very wet soils or detritus-rich soils may require less sample by mass. Ensure that there is 250–500 µL of empty space in the tube. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

MP Bio's Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep instrument. The use of other products with the FastPrep instrument is not recommended and may result in sample loss or instrument failure. A single 40 second run at a speed setting of 6.0 in the FastPrep instrument is sufficient to lyse almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix E tube for at least 2 minutes between successive FastPrep instrument homogenizations to prevent overheating the sample and tube.

MATERIALS

FastDNA SPIN Kit materials (50 preps; 116560200**)**

- Lysing Matrix E (50 x 2 mL tubes)
- Sodium Phosphate Buffer (60 mL)
- MT Buffer (8 mL)
- PPS Solution (15 mL)
- Binding Matrix (2 x 30 mL)
- SPIN Modules (50 each)
- Catch Tubes (50 each)
- COncentrated SEWS-M (12 mL)
- DES (20 ml)
- BBS Gel Loading Dye (200 µL)
- User Manual
- Detailed Protocol
- Certificate of Analysis

User supplied materials:

- FastPrep instrument
- Microcentrifuge to spin 2.0 mL tubes
- Microcentrifuge tubes (2.0 mL and 1.5 mL)
- Clean 15 mL tubes for DNA binding
- Rotator or low-speed vortex

SAFETY WARNINGS

Binding Matrix contains components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucous membranes (gloves, lab coat, and eye protection).

PREPARE the sample

1 **ADD** up to 500 mg of soil sample,
978 µL Sodium Phosphate Buffer, and 122 µL MT Buffer to Lysing Matrix E tube

HOMOGENIZE with the FastPrep

2 **LOAD** tube in FastPrep instrument. 10m 40s

PROCESS 00:00:40 at a speed setting of **6.0 m/s.**

CENTRIFUGE at 14000 x g, 00:10:00 to pellet debris

PRECIPITATE proteins

3 **TRANSFER** supernatant to a clean 2 mL microcentrifuge tube. 5m

ADD 250 µL PPS and mix 10 times.

CENTRIFUGE at 14000 x g, 00:05:00 to pellet precipitate.

ADJUST binding conditions

4 **TRANSFER** supernatant to 15 mL tube. 5m

ADD 1 mL Binding Matrix Solution. Invert 00:02:00 and place tube on a rack for 00:03:00

DISCARD 500 µL of supernatant.

BIND the DNA

5 **TRANSFER max** 600 µL of DNA Solution to a SPIN Filter Tube. 1m

CENTRIFUGE at 14000 x g, 00:01:00.
Empty catch tube.

Repeat step 5 if the volume of the mixture is higher than 600 µL.

WASH the SPIN Filter

6 **ADD** 500 µL prepared SEWS-M Solution. 1m

CENTRIFUGE at 14000 x g, 00:01:00.
Empty catch tube.

DRY the SPIN Filter

7 **CENTRIFUGE** again at 14000 x g, 00:02:00. 7m

AIR DRY SPIN Filter for 00:05:00 at Room temperature.

ELUTE the DNA

8 **ADD** 50 µL - 100 µL DES Elution Solution. 1m

CENTRIFUGE at 14000 x g, 00:01:00.
DNA in the catch tube is ready-to-use.