Instruction Manual

FastDNA® SPIN Kit for Soil

Rapid Isolation of PCR - Ready Genomic DNA from Soil Samples Using the FastPrep® System

- > One Call
- > One Source
- > A World of Biotechnology Reagents

Size: 50 preps

Storage:

Ambient temperature $(15 - 30^{\circ}C)$

Catalog # 6560-200

Revision # 6560-200-07DEC

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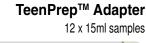
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The FastPrep-24 instrument is delivered with the QuickPrep™ Adapter



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1. Introduction to the FastDNA® SPIN Kit for Soil and the FastPrep® Instruments

The FastDNA® 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, fungi 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 an extremely 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 the 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 together 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 a silica-based GENECLEAN® procedure using SPIN filters. Eluted DNA is ready for PCR, restriction digest, electrophoresis and any other desired application.

2. Kit Components and User Supplied Materials

2.1 FastDNA® SPIN Kit for Soil Components

50x 2.0 ml tubes Lysing Matrix E Sodium Phosphate Buffer 60 ml MT Buffer 8 ml PPS Solution 25 ml Binding Matrix 66 ml SPIN Modules 50 each Catch Tubes 50 each Concentrated SEWS-M 12 ml DES 20ml BBS Gel Loading Dye 200 µl User manual 1 each **MSDS** 1 each Certificate of Analysis 1 each

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FastDNA® Spin Kit for Soil

2.2 User Supplied Materials

FastPrep® Instrument (see Section 9)
Microcentrifuge that can freely 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

3. Important Considerations Before Use

3.1 Preparation of SEWS-M Wash Solution

The FastDNA® SPIN Kit for Soil contains a bottle with 12 ml of Concentrated SEWS-M Wash Solution. Before using this solution, add 100 ml of 100% ethanol and mark on the bottle label the date ethanol was added. Ensure that the bottle is securely closed to prevent evaporation, mix and store at room temperature.

3.2 Sample Lysis with the FastPrep® Instrument

The fill volume in the lysing matrix tube after the addition of the Sodium Phosphate and MT Buffers to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. MP Biomedicals recommends using 500 mg of starting material as long as there is between 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 Biomedicals' 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.

4. Safety Precautions

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 mucus membranes (gloves, lab coat, and eye protection). Consult the enclosed Material Safety Data Sheet for additional details.

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5. Protocol

- 1. Add up to 500 mg of soil sample to a Lysing Matrix E tube. NOTE: See section 3.2 for important guidelines.
- 2. Add 978 µl Sodium Phosphate Buffer to sample in Lysing Matrix E tube.
- 3. Add 122 µl MT Buffer.
- 4. Homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0.
- 5. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.
 - NOTE: Extending centrifugation to 15 minutes can enhance elimination of excessive debris from large samples, or from cells with complex cell walls.
- 6. Transfer supernatant to a clean 2.0 ml microcentrifuge tube. Add 250 μl PPS (Protein Precipitation Solution) and mix by shaking the tube by hand 10 times.
- 7. Centrifuge at 14,000 x g for 5 minutes to pellet precipitate. Transfer supernatant to a clean 15 ml tube. NOTE: While a 2.0 ml microcentrifuge tube may be used at this step, better mixing and DNA binding will occur in a larger tube.
- 8. Resuspend Binding Matrix suspension and add 1.0 ml to supernatant in 15 ml tube.
- 9. Place on rotator or invert by hand for 2 minutes to allow binding of DNA. Place tube in a rack for 3 minutes to allow settling of silica matrix.
- 10. Remove and discard 500 µl of supernatant being careful to avoid settled Binding Matrix.
- 11. Resuspend Binding Matrix in the remaining amount of supernatant. Transfer approximately 600 µl of the mixture to a SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. Empty the catch tube and add the remaining mixture to the SPIN™ Filter and centrifuge as before. Empty the catch tube again.
- 12. Add 500 μ l prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip.

NOTE: Ensure that ethanol has been added to the Concentrated SEWS-M. See section 3.1.

FastDNA® Spin Kit for Soil

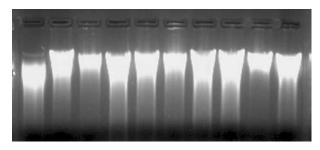
- 13. Centrifuge at 14,000 x g for 1 minute. Empty the catch tube and replace.
- 14. Without any addition of liquid, centrifuge a second time at 14,000 x g for 2 minutes to "dry" the matrix of residual wash solution. Discard the catch tube and replace with a new, clean catch tube.
- 15. Air dry the SPIN™ Filter for 5 minutes at room temperature.
- 16. Gently resuspend Binding Matrix (above the SPIN filter) in 50-100 μl of DES (DNase/ Pyrogen-Free Water).

NOTE: To avoid over-dilution of the purified DNA, use the smallest amount of DES required to resuspend Binding Matrix pellet.

NOTE: Yields may be increased by incubation for 5 minutes at 55°C in a heat block or water bath.

17. Centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the clean catch tube. Discard the SPIN filter. DNA is now ready for PCR and other downstream applications. Store at -20°C for extended periods or 4°C until use.

6. Example Data: DNA Isolation from various Soil Samples and Gel Electrophoresis



DNA from various soil samples extracted with the FastDNA® SPIN Kit for Soil. 20% of the DNA isolated from 500mg soil was loaded on a 1.2% agarose gel (0.5X TAE). Soil was taken from:

Lane 1: tomato pot; Lane 2: sludge;

Lane 3: sandy soil; Lane 4: under pine tree;

Lane 5: under palm tree; Lane 6: green garden;

Lane 7: Nile Lilly pot; Lane 8: lawn grass;

Lane 9: citrus tree; Lane 10: avocado tree. DNA ranges from 4-20 kb.



7. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA® SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals, Santa Ana,CA).

8. References

Soil -

Jacob Bælum et al. (2006). Appl. Envir. Microbiol., Vol 72: 1476 - 1486.

Sediment -

Tracy J. Mincer et al. (2005). Appl. Envir. Microbiol., Vol 71: 7019 - 7028.

Snow samples -

Takahiro Segawa et al. (2005). Appl. Envir. Microbiol., Vol 71: 123 - 130.

Feces -

Alice Layton et al. (2006). Appl. Envir. Microbiol., Vol 72: 4214 - 4224

Sediments and Soil -

Francis C.A. et al. (2005). PNAS. Vol 102: 14683 - 14688

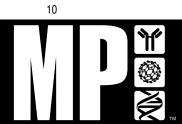
Soil -

Akira Ando et al. (2005). Appl. Envir. Microbiol., Vol. 71: 7075-7082



9. Related Products

Description	Size	Catalog #
FastPrep® 24 Instrument	100-230V	6002-500
FastPrep® FP100A Instrument	100V	6001-100
FastPrep® FP120A Instrument	120V	6001-120
FastPrep® FP220A Instrument	220V	6001-220
FastDNA® Kit	100 preps	6540-400
FastDNA® SPIN Kit	100 preps	6540-600
FastRNA® Pro Soil-Direct Kit	50 preps	6070-050
FastRNA® Pro Soil-Indirect Kit	50 Preps	6075-050
FastRNA® Pro Red Kit (Yeast & Fungus)	50 preps	6035-050
FastRNA® Pro Green Kit (Plant & Animal)	50 preps	6045-050
FastRNA® Pro Blue Kit (Bacteria)	50 preps	6025-050
FastProtein™ Blue Matrix	50 preps	6550-400
FastProtein™ Red Matrix	50 preps	6550-600
Lysing Matrix E	50 x 2 ml tubes	6914-050
Lysing Matrix E	100 x 2 ml tubes	6914-100
Lysing Matrix E	500 x 2 ml tubes	6914-500



10. Product Use Limitation & Warranty

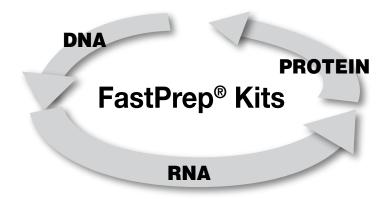
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Ready-to-use Protocols For DNA, RNA And Protein Isolation From Any Sample

- Rapid and reproducible sample lysis and purification process
- No cross contamination with the closed lysing matrix tubes
- · Increased yields of high quality DNA, RNA and proteins
- · Integrity and size of DNA, RNA and proteins are retained
- Nucleic acids and proteins are ready-to-use in downstream application



FastDNA® Kit and FastDNA® Spin Kit

Cat N° 6540-400 - Cat N° 6540-600 respectively (100 preps)

- · Lyse and isolate DNA in less than 30 minutes
- Plant, animal, yeast, fungal and microbial samples
- No hazardous organic reagents required
- SPIN filters streamline silica handling (FastDNA Spin Kit)

FastDNA® Spin Kit for Soil

Cat N° 6560-200 (100 preps)

- Lyse and isolate DNA in less than 30 minutes
- Variety of soil and environmental sample types
- · No hazardous organic reagents required
- · SPIN filters streamline silica handling

FastRNA® Pro Blue Kit

Cat N° 6025-050 (50 preps)

- · For use with gram positive and gram negative bacteria
- Lyse up to 10¹⁰ cells per 2ml tube
- · Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Red Kit

Cat N° 6035-050 (50 preps)

- For use with yeast cells and fungal tissue
- Lyse up to 10¹⁰ cells per 2ml tube
- · Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Green Kit

Cat N° 6045-050 (50 preps)

- For use with all plant and animal samples
- Lyse 50-100 mg tissue per 2ml tube
- · Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Soil-Direct Kit and FastRNA® Pro Soil-Indirect Kit

Cat N° 6070-050 - Cat N° 6075-050 respectively (50 preps)

- Isolate RNA from soil samples (direct kit) and washed soil (indirect kit) in less than 2 hours
- Variety of soil and environmental sample types
- RNA protected during and after processing
- Humic acids reduced to allow uninhibited RT-PCR
- · Includes additional reagents for even further purification if necessary
- SPIN filters streamline silica handling

FastProtein™ Blue Matrix

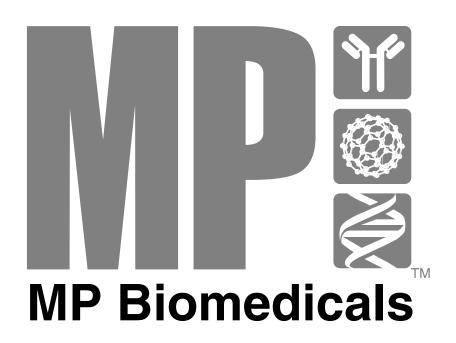
Cat N° 6550-400 (50 preps) - Cat N° 6550-500 (100 preps)

- Release of proteins from gram positive and gram negative bacteria in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- · Ideal for optimizing induction conditions

FastProtein™ Red Matrix

Cat N° 6550-600 (50 preps) - Cat N° 6550-700 (100 preps)

- Release of proteins from yeast cells and fungi in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- Ideal for optimizing induction conditions



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QuickPrep™ Adapter

24 x 2 ml samples



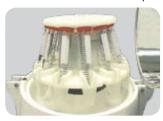
HighPrep™ Adapter

48 x 2ml samples



TeenPrep™ Adapter

12 x 15ml samples



BigPrep[™] Adapter 2 x 50 ml samples



The FastPrep-24 instrument is delivered with the QuickPrep™ Adapter



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FastDNA® SPIN Kit for Soil

Protocol Revision # 6560-200-07DEC



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