



Instruction Manual

FastDNA[®] SPIN Kit for Feces

*Rapid Isolation of Genomic DNA from Human and
Animal Stool Samples Using the FastPrep[®] System*

› *One Call*

› *One Source*

› *A World of
Biotechnology
Reagents*



50 Preps

Storage:
Ambient temperature (15 – 30°C)

Catalog # 6570-200

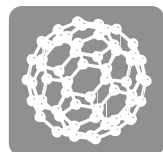
Revision # # 6570-200-10JUN

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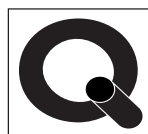
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1. Introduction to the FastDNA® SPIN Kit for Feces and the FastPrep® Instruments

The FastDNA® SPIN Kit for Feces quickly and efficiently isolates PCR-ready genomic DNA from fresh or frozen human and animal stool samples. Designed for use with the FastPrep® Instruments from MP Biomedicals; host cells as well as bacteria, fungi, viruses, protists and other cells present in fecal samples 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 stool organisms. Stool samples are pre-treated by washing in a Pre-Lysis Buffer. Homogenization in the FastPrep® Instrument with Lysing Matrix E takes place in the presence of MT Buffer and Sodium Phosphate Buffer. The pre-lysing and lysing reagents were carefully developed to eliminate contaminants and to protect and solubilize nucleic acids upon cell lysis. These reagents work together to facilitate extraction of genomic DNA with minimal RNA or humic acid contamination. Following lysis, samples are centrifuged to pellet debris and lysing matrix. DNA is purified from the supernatant with a silica-based GENECLAN® procedure using SPIN filters. Eluted DNA is ready for digestion, electrophoresis, PCR and any other desired application.

2. Kit Components and User Supplied Materials

2.1 FastDNA® SPIN Kit for Feces Components

Lysing Matrix E	50x 2.0 ml tubes
PLS Solution	25 ml
MT Buffer	8 ml
Sodium Phosphate Buffer	100 ml
PPS	25 ml
Binding Matrix	66 ml
Wash Buffer #1	120 ml
Wash Buffer #2	12 ml
TES	20 ml
BBS Gel Loading Dye	200 ul
SPIN Filter Tubes	50 each
Catch Tubes	50 each
User manual	1 each
MSDS (Online: www.mpbio.com)	1 each
Certificate of Analysis	1 each

www.mpbio.com

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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)
Sterile 15 ml tubes for DNA binding
Rotator or low-speed vortex
100% Ethanol

3. Important Considerations Before Use

3.1 Preparation of Wash Buffer #2

The FastDNA® SPIN Kit for Feces contains Wash Buffer #2, which is a plastic screw-top bottle containing 12 ml of a concentrated salt wash solution. Before using this buffer, 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, 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 Buffer Solution 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.

3.3 Recovery of DNA from Dry Samplest

To optimize DNA recovery from extremely dry samples, stool samples should be solubilized in Sodium Phosphate Buffer in a separate tube. Weigh approximately 500 mg of dry stool and add an equivalent volume of buffer (~500 ul). Vortex the sample at a low speed to create a homogeneous solubilized stool sample. Transfer 500 mg of the solubilized sample to the Lysing Matrix E tube and process as normal

4. Safety Precautions

PLS Solution, Binding Matrix and Wash Solution #2 contain 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 Material Safety Data Sheet at www.mpbio.com for additional details.

5. Protocol

1. In a 2 ml **Lysing Matrix E** tube, add 500 mg grams feces, 825 μ l **Sodium Phosphate Buffer**, and 275 μ l of PLS solution. Shake to mix then vortex 10-15 seconds

NOTE: See section 3.3 for other important guidelines.

2. Centrifuge samples at 14,000 x g for 5 minutes and decant supernatant.
3. Add 978 μ l **Sodium Phosphate Buffer** and 122 μ l **MT Buffer**. Shake vigorously or vortex briefly to mix.
4. Homogenize samples in the FastPrep® 24 instrument at setting 6.0m/s for 40 seconds.
5. Centrifuge samples at 14,000 x g for 5 minutes.

NOTE: Extending centrifugation to 15 minutes can enhance elimination of excessive debris from large samples, or from cells with complex walls.

6. Transfer the supernatant to a clean 2.0 ml centrifuge tube.

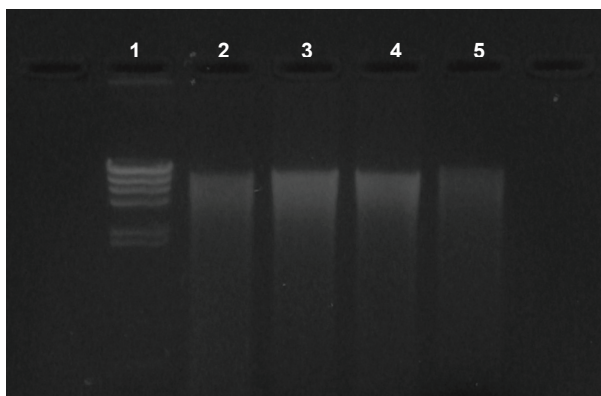
7. Add 250 µl of **PPS** solution, shake vigorously to mix, and incubate at 4°C for 10 minutes. Do not vortex! Centrifuge samples at 14,000 x g for 2 minutes.
 8. While samples are centrifuging, add 1 ml of **Binding Matrix Solution** to a clean 15 ml conical tube (not supplied).
 9. Transfer supernatant to the **Binding Matrix Solution** in the 15 ml conical. Shake gently by hand to mix, then place on a shaker/rocker for 3-5 minutes.
 10. Centrifuge samples at 14,000 x g for 2 minutes. Decant the supernatant.
 11. Wash the binding mixture pellet by gently resuspending with 1 ml **Wash Buffer #1**.
 12. The following step will require two spins. First, transfer approximately 600 µl of the binding mixture to a SPIN Filter tube and centrifuge at 14,000 x g for 1 minute. Empty the catch tube and add the remaining binding mixture to the SPIN Filter tube and centrifuge as before. Empty the catch tube again.
 13. Add 500 µl of prepared **Wash Buffer #2** to the SPIN Filter tube and gently resuspend using the force of the liquid from the pipette tip to resuspend the pellet. Do not vortex!
- NOTE: Ensure that ethanol has been added to the Wash Buffer #2. See section 3.1.
14. Centrifuge samples at 14,000 x g for 2 minute. Discard the flow-through.
 15. Centrifuge the sample again for 2 minutes to extract residual ethanol from the binding matrix and dry the sample.
 16. Transfer the SPIN Filter bucket to a clean 1.9 ml Catch Tube. Add 50-100 µl **TES**. Flick the tube or stir the matrix with a pipette tip to resuspend the pellet. Do not vortex!
 17. Centrifuge samples at 14,000 x g for 2 minutes to elute purified 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. Troubleshooting

6.1 Humic Acid Contamination

Some stool samples contain very high levels of humic acids which may co-purify with the genomic DNA. PCR applications are hindered by the presence of humic acid. If the final eluted DNA returns a negative PCR result, then humic acid contamination might be the reason. The FastDNA® SPIN Kit for Feces is designed to eliminate humic acid contamination in feces. If humic acid contamination is suspected, repeat the protocol from the beginning with a fresh stool sample and repeat step #1 once, as well as steps #10 & #11 once each in order.

7. Example Data: DNA Isolation from Animal Stool Samples and Gel Electrophoresis



DNA from stool samples extracted with the FastDNA® Kit for Feces. Approximately 200 ng of isolated DNA was loaded on a 1.2% agarose gel (0.5X TAE). Lane 1: Lambda Hind III marker; Lane 2: 500 mg bovine stool; Lane 3: 500 mg equine stool; Lane 4: 500 mg feline stool; Lane 5: 500 mg avian stool.

8. Recommended Reference Format for Publications

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

9. References

Ando A, et al. (2005). Appl. Envir. Microbiol. Nov;71(11):7075-82.

Bælum J, et al. (2006). Appl. Envir. Microbiol. Feb; 72(2):1476-86.

Braid MD, et al. (2003). J Microbiol Methods. Mar;52(3):389-93.

Dong D, et al. (2006). J Microbiol Methods. Aug;66(2):217-22.

Kuntz RL, et al. (2004). Water Res. Sep;38(16):3551-7.

Layton A, et al. (2006). Appl. Envir. Microbiol. Jun;72(6):4214-24.

10. Related Products

Description	Size	Catalog #
FastPrep® 24 Instrument	100-230V	6004-500
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
FastDNA® SPIN Kit for Soil	50 preps	6560-200
FastDNA® 50ml SPIN Kit for Soil	10 preps	6570-200
FastRNA® Pro Soil-Direct Kit	50 preps	6070-050
FastRNA® Pro Soil-Indirect Kit	50 preps	6075-050

Description	Size	Catalog #
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

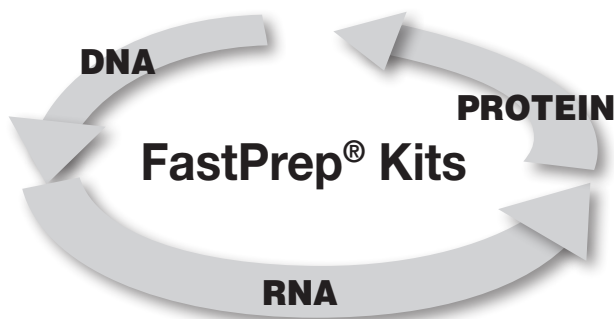
11. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification.

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Take Advantage of FastPrep® Kits 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 10^{10} 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 10^{10} 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

Convenient Lysing Matrix Tubes for Every Need

MP Biomedicals guarantees the BEST performance from your FastPrep®-24 Instrument when used in combination with FastPrep Lysing Matrix Tubes



Lysing Matrix A

Each impact-resistant 2 ml tube contains garnet matrix and one 1/4 inch ceramic sphere. Extra 1/4 inch ceramic spheres are packaged separately. Lysing Matrix A tubes have orange caps and are found in the FastDNA® and FastDNA® SPIN Kits. Lysing Matrix A is used for all sample types except soil for the subsequent isolation of genomic DNA.



Lysing Matrix B

Each impact-resistant 2 ml tube contains 0.1 mm silica spheres. Lysing Matrix B tubes have blue caps and are found in the FastRNA® Pro Blue Kit and FastProtein™ Blue Matrix. Lysing Matrix B is used for lysis of gram positive and gram negative bacteria.



Lysing Matrix C

Each impact-resistant 2 ml tube contains 1 mm silica spheres. Lysing Matrix C tubes have red caps and are found in the FastRNA Pro Red Kit and FastProtein Red Matrix. Lysing Matrix C is used for lysis of yeast and fungi.



Lysing Matrix D

Each impact-resistant 2 ml tube contains 1.4 mm ceramic spheres. Lysing Matrix D tubes have green caps and are found in the FastRNA® Pro Green Kit for isolation of total RNA from plants and animals.



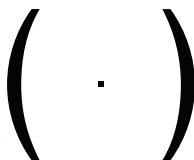
Lysing Matrix E

Each impact-resistant 2 ml tube contains 1.4 ceramic spheres, 0.1 mm silica spheres, and one 4 mm glass bead. Lysing Matrix E tubes have purple caps and are found in the FastDNA® SPIN Kit for Soil and the FastRNA® Pro Soil Kits.

Dirty Samples?



**Tiny (or Huge)
Samples?**



Tough Samples?



No Problem with FastPrep.



Higher yields. Consistent quality. Super fast.

FastPrep lets you get the most usable DNA, RNA and proteins from even your most difficult samples, fast and easy, every time.

FREE fastprep DEMO!

Learn more at:
www.mpSamplePrep.com

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Instruction Manual

FastDNA® SPIN Kit for Feces

Protocol Revision # 6570-200-10JUN

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