Instruction Manual

FastDNA™ SPIN Kit for Feces

Rapid isolation of genomic DNA from human and animal stool samples using the FastPrep® System

Size: 50 preps/Sample Kit: 5 preps

Storage: Ambient Temperature – 15-30 °C

Cat. No.: 116570200/116570000

Protocol Revision: #116570200-201912/#116570000-201912

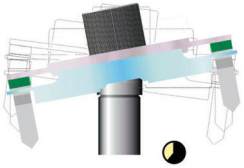
MSDS now available online

MP

www.mpbio.com

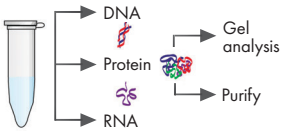
Select the Best Homogenization and Extraction Solution for Your Application.

HOMOGENIZATION



1 Minute

EXTRACTION



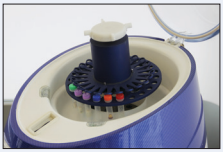
Instruments

FastPrep-24™ 5G

FastPrep-96™

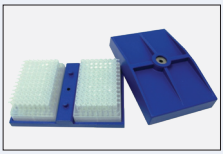
Super FastPrep-2™

Adapters



Metal

Cryogenic



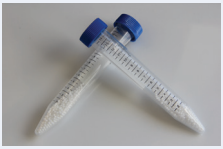
High Throughput

Large Sample Volume

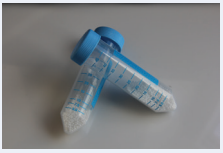
Lysing Matrix Tubes

2 mL

4.5 mL



15 mL



50 mL

Extraction Kits

DNA

RNA

Protein

FastDNA™ SPIN Kit For Feces

Rapid isolation of genomic DNA from human and animal stool samples using the FastPrep® System

- **Size:** 50 preps/Sample Kit: 5 preps
- **Storage:** Ambient Temperature – 15-30 °C
- **Cat. No.:** 116570200/116570000
- **Protocol Revision:** #116570200-201912/#116570000-201912

Table of Contents

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

The FastDNA™ SPIN Kit for Feces quickly and efficiently isolates PCR-ready genomic DNA directly 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 pretreated by washing in a Pre-Lysis Buffer. 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 or humic acid contamination.

Following lysis, samples are centrifuged to pellet 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 Feces Components

Product	Kit Size	Cat. No.	Sample Kit Size	Sample Cat. No.
Lysing Matrix E	50 x 2.0 mL tubes	116914050	5 x 2.0 mL tubes	116914005
Sodium Phosphate Buffer	100 mL	116570205	10 mL	116570005
MT Buffer	8 mL	116511202	650 µL	116570002
PLS Solution	25 mL	116570201	1.5 mL	116570001
PPS Solution	25 mL	116560203	1.5 mL	116570003
Binding Matrix	66 mL	116540408	5 mL	116570008
Wash Buffer #1	120 mL	116570209	5 mL	116570009
Wash Buffer #2	12 mL	116570204	750 µL	116570004
TES	20 mL	116570206	1 mL	116570006
Spin Filter Tubes	50 each	116560210	5 each	116560010
Catch Tubes	50 each	116560211	5 each	116560011
User manual	1 each	-	1 each	-
Certificate of Analysis	1 each	-	1 each	-

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 *(sample kit: 1.2 mL)* of a concentrated salt wash solution. Before using this buffer, add 100 mL *(sample kit: 10 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 of the lysing matrix tube after addition of 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 up to 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 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.

3.3 Recovery of DNA from Dry Samples

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 µL). 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). Please consult the **Material Safety Data Sheet** found online at www.mpbio.com for additional details.

5. Protocol

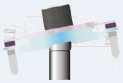
5.1 FastDNA™ SPIN Kit for Feces Typical Work Flow

1. Prepare the Sample

875 µL Sodium Phosphate Buffer.
275 µL PLS Solution.
Shake to mix, vortex 10-15 sec.
Centrifuge and discard supernatant.
Add 978 µL Sodium Phosphate Buffer, 122 µL MT Buffer and shake.

2. Homogenize with the FastPrep instrument *(or similar instrument)*

40 sec



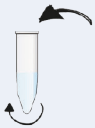
Load tube in FastPrep instrument.
Process: 40 s at a speed setting of 6.0 m/s.

5 mins
14,000 g

Centrifuge to pellet debris.

3. Precipitate proteins

2 mins
14,000 g



Transfer supernatant to a clean 2 mL microcentrifuge tube. Add 250 µL PPS and mix. Incubate at 4°C for 10 mins.

Do not vortex.

4. Adjust binding conditions

15 mL tube

2 mins
14,000 g

Transfer supernatant to 15 mL tube.
Add 1 mL Binding Matrix Solution.
Shake 3-5 mins.
Centrifuge and discard supernatant.
Wash with 1 mL of Wash Buffer #1

5. Bind the DNA

2 mins
14,000 g

Transfer max 600 µL of DNA Solution to a SPIN™ Filter Tube. Empty catch tube.
Repeat step 5 if the volume of the mixture is higher than 600 µL.

6. Wash the SPIN™ Filter

2 mins
14,000 g

Add 500 µL of Wash Buffer #2.
Resuspend gently.
Do not vortex.
Centrifuge and discard the flow through.

7. Dry the SPIN™ Filter

2 mins
14,000 g

Centrifuge 14,000 g for 2 mins.

8. Elute the DNA

Use a new catch tube

2 mins
14,000 g

Add 60-100 µL TES Elution Solution.
Do not vortex.
Centrifuge to elute.
DNA in the catch tube is ready to use.

5.2 FastDNA™ SPIN Kit for Feces Detailed Protocol

NOTE: See section 3 for other important guidelines

1. In a 2 mL Lysing Matrix E tube, add 500 mg feces sample, 825 µL Sodium Phosphate Buffer, and 275 µL of PLS solution. Shake to mix. Vortex 10-15 seconds

2. Centrifuge samples at 14,000 x g for 5 minutes and decant supernatant.

3. Add 978 µL Sodium Phosphate Buffer and 122 µL 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. 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 60-100 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. Table of Recommended FastPrep® Settings

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Soil and Sediment					
Sediment	Soil/Rock	50 mg	E	5.5	2 x 30 sec
Soil	Sandy Sample	50 mg	E	4.0	4 x 30 sec
Soil	Litter	50 mg	E	5.5	30 sec
Soil	Brunisol Dark Gray Luvisol	50 mg	E	5.5	40 sec
Soil	Soil from Grassland	50 mg	E	5.5	2 x 30 sec
Soil	Rhizosphere	50 mg	E	6.0	40 sec
Sediment	Marine Sediment	50 mg	E	5.5	2 x 40 sec
Soil	Asphalt-permented Soil	50 mg	E	6.0	40 sec
Bacteria					
Listeria monocytogenes	Cells	10^9 cells	B	6.0	3 x 30 sec
Streptococcus pyogenes	Cells	10^9 cells	B	6.0	20 sec
Streptococcus mutants	Cells	10^9 cells	B	6.0	30 sec
Streptococcus aureus	Cells	10^9 cells	B	6.0	2 x 40 sec
Photorhabdus luminescens	Cells	10^9 cells	B	6.0	2 x 40 sec
Escherichia coli	Cells	10^9 cells	B	6.0	30 sec
Mycobacterium tuberculosis	Cells	10^8 cells	B	6.0	2 x 45 sec
Lactococcus lactis	Cells	10^8 cells	B	6.0	3 x 30 sec
Yeast & Fungi					
Saccharomyces cerevisiae	Cells	2 x 10^8 cells	C	6.0	40 sec
Schizosaccharomyces pombe	Cells	10^8 cells	C	5.0	4 x 15 sec
Candida albicans	Cells	10^8 cells	C	6.0	2 x 30 sec
Cryptococcus neoformans	Cells	10^8 cells	C	6.0	4 x 30 sec
Aspergillus fumigatus	Cells	10^8 cells	C	6.0	2 x 30 sec
Fusarium solani	Cells	10^8 cells	C	6.0	2 x 30 sec

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Plants					
Alpowa Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Alpowa Wheat	Seed	100 mg	A	6.0	40 sec
Arabidopsis thaliana	Fresh Leaves	50 mg	D	6.0	40 sec
Arabidopsis thaliana	Fresh Leaves	200 mg	D	6.0	2 x 40 sec
Bartlett Pear	Leaf Tissue	50 mg	D	6.0	40 sec
Classic Oat	Leaf Tissue	75 mg	D	6.0	40 sec
Classic Oat	Seed	100 mg	A	6.0	40 sec
Corn	Leaf Tissue	100 mg	D	6.0	40 sec
Crest Barley	Leaf Tissue	100 mg	D	6.0	40 sec
Crest Barley	Root	300 mg	A	6.0	40 sec
Kaybonnet Rice	Leaf Tissue	100 mg	D	6.0	40 sec
Kaybonnet Rice	Seed	100 mg	A	6.0	40 sec
Klages Barley	Root	300 mg	A	6.0	40 sec
Klages Barley	Leaf Tissue	70 mg	D	6.0	40 sec
Tobacco	Leaf Tissue	75 mg	D	6.0	40 sec
Laffite Rice	Leaf Tissue	75 mg	D	6.0	40 sec
Laffite Rice	Sprout Leaf	100 mg	D	6.0	2 x 30 sec
Soybean	Seed	100 mg	A	6.0	40 sec
Corn	Seed	100 mg	A	6.0	40 sec
Oat FL 502	Leaf Tissue	75 mg	D	6.0	40 sec
Oat FL 502	Seed	100 mg	A	6.0	40 sec
Riser Oat	Leaf Tissue	70 mg	D	6.0	40 sec
Richland Soybean	Leaf Tissue	100 mg	D	6.0	40 sec
Tam Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Tam Wheat	Root	80 mg	A	6.0	40 sec
Tomato, Early Girl	Leaf Tissue	75 mg	D	6.0	4 x 30 sec
Williams 82 Soybean	Leaf Tissue	70 mg	D	6.0	40 sec
Wrens Rye	Leaf Tissue	100 mg	D	6.0	40 sec
Pine	Needle	100 mg	A	6.0	40 sec

7. Troubleshooting

7.1 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

8. Related Products

Product Name	Cat. No.
Instruments	
FastPrep-24™ Classic	116004500
FastPrep-24™ 5G	116005500
FastPrep-96™	116010500
SuperFastPrep-2™	116012500
MPure-12™	117002200
Kits	
FastDNA™ Kit	116540400
FastRNA™ Pro Blue Kit (Bacteria)	116025050
FastDNA™ SPIN Kit	116540600
FastDNA™ SPIN Kit for Soil	116560200
FastRNA™ Pro Soil-Direct Kit	116070050
FastRNA™ Pro Soil-Indirect Kit	116075050
FastRNA™ Pro Green Kit (Plant & Animal)	116045050
FastRNA™ Pro Red Kit (Yeast & Fungus)	116035050
FastPROTEIN™ Blue Matrix	116550400
FastPROTEIN™ Red Matrix	116550600

9. References

Strati, F.; Di Paola, M.; Stefanini, I.; Albanese, D.; Rizzetto, L.; Lionetti, P.; Calabrò, A.; Jousson, O.; Donati, C.; Cavalieri, D.; et al. *Front. Microbiol.* **2016**, *7*, DOI 10.3389/fmicb.2016.01227.

Qiu, X.; Zhang, F.; Yang, X.; Wu, N.; Jiang, W.; Li, X.;. Li, X; Liu, Y. *Scientific Reports.* **2015**, *5*, 10416.

Watanabe, K.; Igarashi, M.; Li, X.; Nakatani, A.; Miyamoto, J.;. Inaba, Y; Sutou, A.; Saito, T.; Sato, T.; Tachibana, N.; et al. *PLOS ONE.* **2018**, *13*, e0202083.

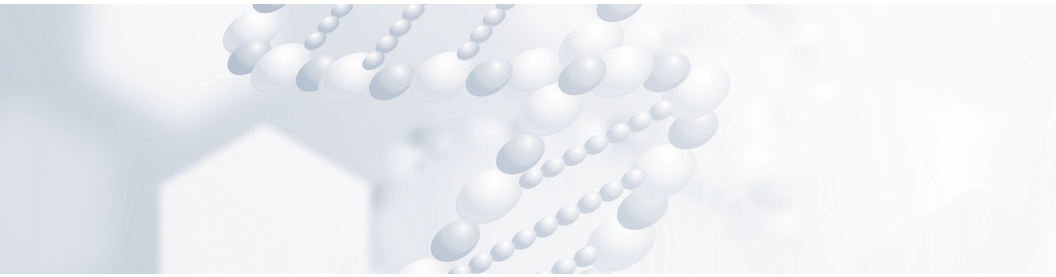
Nale, J.; Redgwell, T.; Millard, A.; Clokie, M.; Nale, J. Y.; Redgwell, T. A.; Millard, A.; Clokie, M. R. J. *Antibiotics.* **2018**, *7*, 13.

Nicolucci, A. C.; Hume, M. P.; Martínez, I.; Mayengbam, S.; Walter, J.; Reimer, R. A. *Gastroenterology.* **2017**, *153*, 711–722.

11. Product Use Limitation and 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. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).



One Call. One Source. A World of Sample Preparation Products.

- Apoptosis
- Cell Biology
- Culture Growth Media
- FastPrep® Sample Prep
- Immunology
- Molecular Biology
- Adsorbents
- Biochemicals
- Fine Chemicals
- Labware
- Dosimetry
- Research Diets
- SafTest™ Food Quality
- Diagnostics
- Drugs of Abuse
- Infectious Disease
- EIA/RIA

MP Biomedicals

Americas: 800.854.0530 | custserv@mpbio.com
Europe: 00800.7777.9999 | custserv.eur@mpbio.com
Japan: 03.6667.0730 | sales.japan@mpbio.com
Singapore: 65.6775.0008 | enquiry_ap@mpbio.com
South Korea: 82.2.425.5991 | info.korea@mpbio.com
Australia: 61.2.8824.2100 | aus.cs@mpbio.com
China: 86.4000.150.0680 | mpchina@mpbio.com
India: 91.22.27636921/22/24 | info.india@mpbio.com
New Zealand: 64.9.912.2460 | nzsales@mpbio.com

LEARN MORE
www.mpbio.com