MagBeads FastDNA® Kit for Feces

Magnetic Beads for Quick Isolation of Genomic DNA from Human and Animal Feces

Size: 50 preps

Storage: 15-30 ˚C, except for Magnetic Beads at 2-8 ˚C

Cat. No.: 116570400

Instruction Manual

Table of Contents

1. Introduction

The MagBeads FastDNA® Kit for Feces allows quick and efficient isolation of high-quality genomic DNA from fresh or frozen human and animal feces in less than 60 mins. Samples are placed into Lysing Matrix E tube and used with FastPrep® Instruments from MP Biomedicals to effectively lyse host cells as well as bacteria, fungi, viruses, protists and other cells present in fecal samples within 40 seconds. The kit is also compatible with most of the automated nucleic acid extraction instruments on the market or it can be operated manually. It consists of specially formulated reagents to eliminate humic acid, polysaccharides, phenolic compounds, and enzyme inhibitors from feces and thus allow extraction of highly pure genomic DNA ready for PCR, restriction digestion, electrophoresis and other desired applications.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysing Matrix E	50 x 2 mL	116914050
Pre-Wash Buffer	60 mL	116570402
Lysis Buffer F1	60 mL	116570403
Lysis Buffer F2	8 mL	116570404
RNase A Solution	1 mL	116570427
PPS (Protein Precipitate Solution)	15 mL	116570405
Binding Buffer F	18 mL	116570406
Magnetic Beads	550 µL	116570407
Wash Buffer F1	15 mL	116570408
Wash Buffer F2	30 mL	116570409
TE Buffer	10 mL	116570410
2.0 mL microcentrifuge tubes	100 ea	116570412
1.5 mL microcentrifuge tubes	50 ea	116570413
Instruction Manual	1 ea	-
Quick-Start Protocol	1 ea	-
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- 100% ethanol (135 mL)
- Isopropanol (42 mL)
- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex
- Microcentrifuge capable of at least 14,000 rpm
- MP Magnetic Rack 8 (Cat. No. 116570426) or Rack 24 (Cat. No. 116570413) or any magnetic rack
- Water baths or heat blocks
- Shaker

3. Storage and Stability

All MagBeads FastDNA® Kit for Feces components are guaranteed for at least 24 months from the date of manufacture when stored as follows. Store Magnetic Beads at 2-8 ˚C. Store all other components at room temperature (15-30 ˚C). Check buffers for precipitation before use. Dissolve any precipitation by warming to 55 ˚C.

4. Notes Before Starting

Please tick as appropriate.

- ☐ Store Magnetic Beads at 2-8 ˚C upon arrival, do not freeze.
- ☐ Expect precipitation in Pre-Wash Buffer and Lysis Buffer 1, warm the solutions to 55 ˚C will dissolve the precipitate.
- ☐ Add 42 mL isopropanol to Binding Buffer F and mark on the bottle label the date isopropanol was added.
- ☐ Add 45 mL 100% ethanol to Wash Buffer F1 and mark on the bottle label the date ethanol was added.
- ☐ Add 90 mL 100% ethanol to Wash Buffer F2 and mark on the bottle label the date ethanol was added.

5. Safety Precautions

Binding Buffer F and **Wash Buffer F1** contain components that when in contact with human tissue, may cause irritation. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucus membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

MagBeads FastDNA® Kit for Feces can be operated manually or using automation. A Quick-Start Protocol is provided in the kit for quick reference throughout the extraction process.

6.1 Manual Extraction

1. In a 2 mL **Lysing Matrix E** tube, add 30-300 mg fecal sample and 1000 µL **Pre-Wash Buffer**. Vortex at maximum speed for 20-40 seconds or until the stool sample is completely homogenized.
   Note: Ensure that there is at least 200 µL of empty space in the tube. For extremely dry or frozen fecal samples, wait for 5mins after adding Pre-Wash Buffer，then vortex to achieve homogeneity.
2. Centrifuge samples at 14,000 rpm for 3-5 mins and decant supernatant.
3. Add 980 µL **Lysis Buffer F1**, 10 µL **RNase A Solution** and 120 µL **Lysis Buffer F2** to sample in Lysing Matrix E tube. Shake to mix.
4. Homogenize sample in FastPrep® Instrument for 40 seconds at a speed setting of 5.0 m/s.
   Note: The speed and time can be changed according to different fecal samples. Vortex 10 mins at maximum speed if FastPrep® Instrument is not available. For low A260/280 samples, heat it at 70 ˚C for 10 mins, then centrifuge.
5. Centrifuge at 14,000 rpm for 5 mins to pellet debris.
   Note: Extending centrifugation to 10 mins can enhance elimination of excessive debris from large samples, or from cells with complex cell walls.
6. Transfer 900 µL supernatant to a clean **2.0 mL microcentrifuge tube**. Add 250 µL **PPS** and mix by inverting the tube 20 times. Do not vortex.
7. Centrifuge at 14,000 rpm for 5 mins to pellet precipitate.
8. Transfer 1000 µL supernatant carefully to a new **2.0 mL microcentrifuge tube**. Add 1000 µL **Binding Buffer F** and 10 µL **Magnetic Beads** to supernatant. Vortex or invert the tube to mix.
   Note: Ensure Magnetic Beads is thoroughly mixed before transferring it to supernatant.
9. Place the tube on shaker for 5-10 mins to allow binding.
10. Place the tube on magnetic rack for 3-5 mins, allow Magnetic Beads to settle, then discard supernatant.
    Note: If the supernatant is too turbid or Magnetic Beads are attracted slowly, extend attraction time.
11. Add 1000 µL **Wash Buffer F1** to the tube and place on shaker for 3 mins.
12. Place the tube on magnetic rack for 1 min, allow Magnetic Beads to settle, then discard supernatant.
13. Add 1000 µL **Wash Buffer F2** to the tube and place on shaker for 3 mins.
14. Place the tube on magnetic rack for 1 min, allow Magnetic Beads to settle, then discard supernatant.
15. Repeat step 13 to step 14 for a second wash step.
16. Air dry Magnetic Beads for 5-10 mins at 55 ˚C by placing the tube on a heat block.
    Note: Make sure Magnetic Beads are completely dry. This is for removal of residual ethanol and residual ethanol may interfere with downstream applications.
17. Add 100 µL **TE Buffer** to resuspend dried Magnetic Beads and incubate on a heat block at 55˚C for 5 mins.
18. Place the tube on a magnetic rack for 3-5 mins until Magnetic Beads settling, transfer supernatant (eluted DNA) into a clean **1.5 mL microcentrifuge tube**. DNA is now ready for PCR and other downstream applications. Store at -20˚C for extended periods.

6.2 Automation Extraction

1. In a 2 mL **Lysing Matrix E** tube, add 30-300 mg fecal sample and 1000 µL **Pre-Wash Buffer**. Vortex at maximum speed for 20-40 seconds or until the stool sample is completely homogenized.
   Note: Ensure that there is at least 200 µL of empty space in the tube. For extremely dry or frozen fecal samples, wait for 5mins after adding Pre-Wash Buffer，then vortex to achieve homogeneity.
2. Centrifuge samples at 14,000 rpm for 3-5 mins and decant supernatant.
3. Add 490 µL **Lysis Buffer F1**, 10 µL **RNase A Solution** and 60 µL **Lysis Buffer F2** to sample in Lysing Matrix E tube. Shake to mix.
4. Homogenize sample in FastPrep® Instrument for 40 seconds at a speed setting of 5.0 m/s.
   Note: The speed and time can be changed according to different fecal samples. Vortex 10 mins at maximum speed if FastPrep® Instrument is not available. For low A260/280 samples, heat it at 70 ˚C for 10 mins, then centrifuge.
5. Centrifuge at 14,000 rpm for 5 mins to pellet debris.
   Note: Extending centrifugation to 10 mins can enhance elimination of excessive debris from large samples, or from cells with complex cell walls.
6. Transfer 500 µL supernatant to a clean **2.0 mL microcentrifuge tube**. Add 100 µL **PPS** and mix by inverting the tube 20 times. Do not vortex.
7. Centrifuge at 14,000 rpm for 5 mins to pellet precipitate.
8. Transfer 400 µL supernatant carefully to well 2 of 96-well plate. Add other liquid into respective well as shown.

Well	Reagents	Volume (µL)
1	Deionized Water	800
	Magnetic Beads	10
2	Sample Supernatant	400
	Binding Buffer F	400
3	Wash Buffer F1	800
4	Wash Buffer F2	800
5	Wash Buffer F2	800
6	TE Buffer	100

9. Run the instrument according to following settings.

Step	Well	Process	Time (s)			Mixing	Temperature
			Mix	Wait	Attract	Speed	(˚C)
1	1	Magnetic Beads Preparation	60	0	120	Medium	RT
2	2	Binding	600	0	150	Medium	RT
3	3	Wash 1	180	0	120	Medium	RT
4	4	Wash 2	180	0	120	Medium	RT
5	5	Repeat Wash 2	180	0	150	Medium	RT
6	5	Drying	0	600	0	-	RT
7	6	Elute	300	0	150	Medium	55

7. Data

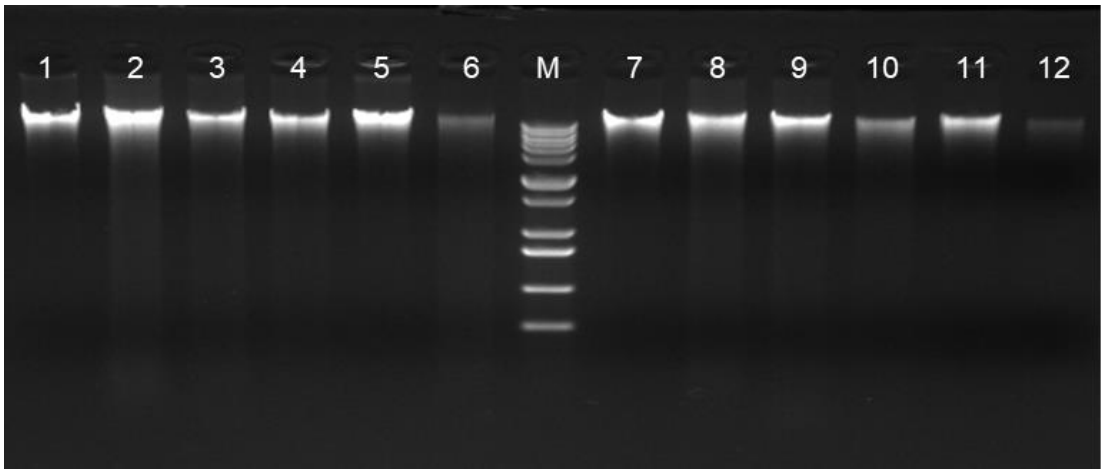

Figure 1: gDNA extracted from different feces sample using MagBeads FastDNA® Kit for Feces.

Lane 1-6: Manual extraction	Lane 7-12: Automation extraction	M: 1kb plus DNA ladder
Lane 1&7: 30 mg swine feces	Lane 2&8: 15 mg mouse feces	Lane 3&9: 30 mg human feces
Lane 4&10: 150 mg chicken feces	Lane 5&11: 150 mg bovine feces	Lane 6&12: 150 mg elephant feces

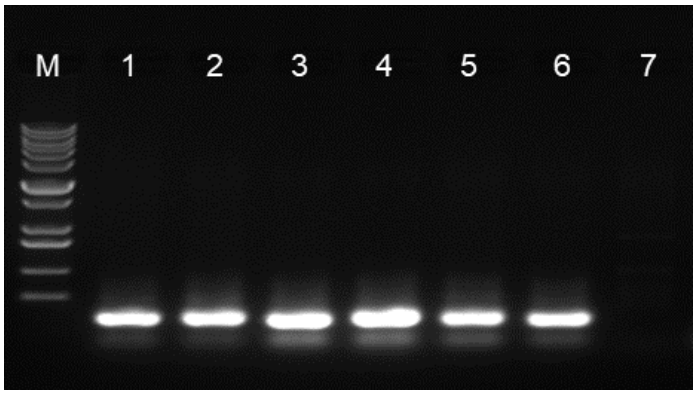

Figure 2: PCR of gDNA extracted from different feces sample using MagBeads FastDNA® Kit for Feces. M: 1kb plus DNA ladder; Lane 1: swine feces; Lane 2: mouse feces; Lane 3: human feces; Lane 4: chicken feces; Lane 5: bovine feces; Lane 6: elephant feces; Lane 7: negative control.

8. Troubleshooting

8.1 Low DNA Yield

1. Make sure extraction as per manual protocol.
2. Low microbiological content: (i) Increase amount of starting material; (ii) Process multiple sample using several Lysing Matrix tubes and then pool the sample.
3. Insufficient lysis: While a FastPrep® speed setting of 5.0 m/s and 40 seconds run time will be adequate for most feces types, additional processing maybe necessary. To repeat processing cycles, it is recommended to incubate Lysing Matrix tubes on ice for 2 mins between cycles to avoid excessive heat buildup.
4. Insufficient Magnetic Beads: Beads are supplied as suspension and must be thoroughly resuspended before aliquoting. Vigorously shake the bottle of Magnetic Beads to produce a uniform suspension, it can be done by vortex too.
5. Magnetic Beads losing binding capacity: Be sure to store Magnetic Beads at 2-8 ˚C upon receiving of kit, do not freeze the beads.
6. Sample loss during transfer: DNA will bind on Magnetic Beads. Make sure all beads are attracted by magnetic rack during transfer.
7. Isopropanol not added to Binding Buffer F： Make sure 42 mL of isopropanol is added to the Binding Buffer F concentrate before use.
8. Poor elution: (i) Make sure the Magnetic Beads is completely resuspended with TE Buffer; (ii) Incubate the Magnetic Beads resuspended with TE Buffer for 5 mins at 55 ˚ C before separation.

8.2 Low A_{260}/A_{280} Ratios for Purified DNA

1. Ethanol not added to Wash Buffer F1 and Wash Buffer F2：Wash Buffers are supplied as concentrate. Make sure correct volume of 100% ethanol is added to the concentrate before use.
2. Proteins not removed efficiently：PPS solution must be efficiently mixed in the lysate (step 6). Invert tube by hand at least 20 times or mix by pipet pumping. Incubate the sample on ice/ keep in fridge for 5 mins can further precipitate proteins from difficult samples.
3. Contaminants not removed efficiently：During the wash with Wash Buffer F1 and Wash Buffer F2, it is necessary to resuspend the Magnetic Beads thoroughly to efficiently remove contaminants. This step should be performed gently and deliberately.
4. Precipitation during washing: If precipitation occurred after addition of Wash Buffer F1, disperse the precipitates by tapping the side of tube or vortex.
5. Heating after homogenization: After homogenization, do not centrifuge down the lysate, heat it at 70 ˚C for 10 mins, this will improve 260/280 of some feces sample.

8.3 High A_{260}/A_{280} Ratios for Purified DNA

Possible RNA contamination, which can be reflected when analyzed using gel electrophoresis. Add RNase A together with Lysis Buffer F1 and Lysis Buffer F2 at step 3.

8.4 Low A_{260}/A_{230} Ratios for Purified DNA

1. Proteins not removed efficiently: Refer to 8.2.2.
2. Contaminants not removed efficiently：Refer to 8.2.3.
3. Residual ethanol in the final eluate: Eluted DNA contaminated with residual ethanol will not freeze when stored at -20 ° C. Increase the air-drying time at step 16 or dry the Magnetic Beads at 55 ° C oven will help to dry out the beads.

8.5 Fragmented DNA

Optimize lysis conditions: High powered bead beating cell disrupters can shear DNA if process settings are too long or powerful. While FastPrep® speed setting of 5.0 m/s and 40 seconds runtime will be adequate for most feces types, it is possible that lowering speed and/or duration settings will result in higher MW DNA.

8.6 DNA Does Not Amplify

1. Quantify DNA yield：By spectrophotometer. High concentration of DNA will inhibit PCR reactions.
2. Dilute DNA template: Inhibitors in the eluted DNA can inhibit PCR reaction. Dilution of template DNA can reduce such inhibition. This should not be necessary with DNA isolated with the MagBeads FastDNA® Kit for Feces but is still an option.
3. Verify PCR optimization conditions：Changing reaction conditions or primer selection may be necessary.
4. Non-specific bands：Check possibility that target DNA is in low abundance in the eluate. It is possible that some species of interest, particularly parasitic cysts and oocytes, may need additional processing or even more aggressive lysing matrix (such as Lysing Matrix A) in order to disrupt the thick protein cell wall.

9. Product Use Limitations & 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). FastDNA®, FastRNA®, FastPrep®, QBiogene®, and BIO 101® Systems are registered trademarks of MP Biomedicals, LLC.

10. Worldwide Ordering and Technical Support

Australia

Tel：+61 2.8824.2100

Tel：+61 1800.249.998

Email：custserv.au@mpbio.com

Austria & Germany

Tel：0800.426.67.337

Email：custserv.de@mpbio.com

Belgium

Tel：00800.7777.9999

Email：custserv.be@mpbio.com

Canada

Tel：+1 800.854.0530

Email：custserv.ca@mpbio.com

China

Tel：+86 400.150.0680

Email：custserv.cn@mpbio.com

Europe

Tel：+33 3.88.67.54.25

Tel：+33 00800.7777.9999

Email：custserv.eur@mpbio.com

France

Tel：+33 3.88.67.54.25

Email：custserv.fr@mpbio.com

India

Tel：+91 22.27636921/22/25

Email：custserv.in@mpbio.com

Italy

Tel：00800.7777.9999

Email：custserv.it@mpbio.com

Japan

Tel：+81 3.6667.0730

Email：custserv.jp@mpbio.com

Latin America

Tel：+1 800.854.0530

Tel：+1 440.337.1200

Email：custserv.la@mpbio.com

New Zealand

Tel：+64 9.912.2460

Email：custserv.nz@mpbio.com

North America

Tel：+1 800.854.0530

Tel：+1 440.337.1200

Email：custserv.na@mpbio.com

Poland

Tel：00800.7777.9999

Email：custserv.po@mpbio.com

Russia

Tel：+7 495.661.0008

Email：custserv.rs@mpbio.com

Serbia

Tel：+381 11.242.1972

Email：custserv.se@mpbio.com

Singapore/APAC

Tel：+65 6775.0008

Tel：+65 6394.7675

Email：custserv.ap@mpbio.com

South Korea

Tel：+82 2.425.5991

Email：custserv.kr@mpbio.com

Switzerland

Tel：00800.7777.9999

Email：custserv.ch@mpbio.com

United Kingdom

Tel：0800.282.474

Email：custserv.uk@mpbio.com

The Netherlands

Tel：00800.7777.9999

Email：custserv.nl@mpbio.com

www.mpbio.com