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
Tel：00800. 7777. 9999
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：+7 495 604-13-44
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

The Netherlands
Tel：00800.7777.9999
Email：custserv.nl@mpbio.com

United Kingdom
Tel：0800.282.474
Email：custserv.uk@mpbio.com

www.mpbio.com

MagBeads FastDNA™ Kit for Soil

Magnetic Beads for Quick Isolation of Genomic DNA from Soil

Size: 50 preps

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

Cat. No.: 116561050

Instruction Manual

Revision 1.0 Mar 2021

Table of Contents

1. Introduction

The MagBeads FastDNA™ Kit for Soil allows quick and efficient isolation of high-quality genomic DNA from soil in less than 60 mins. Samples are placed into Lysing Matrix E tubes 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 soil 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 soil and thus allows for 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 ea	116914050
Lysis Buffer S1	60 mL	116530051
Lysis Buffer S2	8 mL	116530052
RNase A Solution	550 µL	116530053
Inhibitor Removal MS	15 mL	116561051
Binding Buffer MS	15 mL	116561052
Magnetic Beads MS	300 µL	116561053
Wash Buffer S	6 mL	116530056
Wash Buffer S	6 mL	116530056
DES Buffer	10 mL	116530057
Instruction Manual	1 ea	-
Quick-Start Protocol	1 ea	-
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- 100% ethanol (100 mL)
- Isopropanol (35 mL)
- 2.0 mL Microcentrifuge tubes (50 pcs); 1.5 mL Microcentrifuge tubes (100 pcs)
- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex
- Microcentrifuge capable of at least 14,000 x g
- 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

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

4. Notes Before Starting

Please check as appropriate.

- ☐ Store Magnetic Beads at 2-8 ℃ upon arrival；do not freeze.
- ☐ Expect precipitation in Lysis Buffer S1；warming the solution to 55℃ will help dissolve the precipitates.
- ☐ Add 35 mL isopropanol to Binding Buffer MS and mark on the bottle.
- ☐ Add 50 mL 100% ethanol to Wash Buffer S and mark on the bottle.
- ☐ Vortex the sample in a Lysing Matrix E tube at maximum speed for 10 mins if a FastPrep® Instrument is unavailable. Secure samples on the vortex through an adapter to ensure homogenization.
- ☐ Centrifugation speed stated in the manual will be a guideline, use the maximum speed available if 14,000 x g is not feasible.

5. Safety Precautions

Lysis Buffer S2 and **Binding Buffer MS** contain components that may cause irritation when in contact with human tissue. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

MagBeads FastDNA™ Kit for Soil 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. Add 100-500 mg soil sample to a **Lysing Matrix E** tube.
   Note: MagBeads FastDNA™ Kit for Soil can extract gDNA from a wide range of soils. The amount of starting material is dependent on the biomass level in the selected soil type. Soil of high biomass, such as flowerpot soil and topsoil, are only required at 100 mg for the extraction process. After adding the sample to the tube, ensure there is still 1/3-1/4 empty space remaining in the tube.
2. Add 980 µL **Lysis Buffer S1**, 120 µL **Lysis Buffer S2** and 10 µL **RNase A Solution** to the sample in the Lysing Matrix E tube and vortex 5-10 seconds to mix.
3. Homogenize sample in a FastPrep® Instrument for 20-40 seconds at a speed setting of 6.0 m/s.
   Note: The speed and time can be changed according to different soil samples. Vortex 5-10 mins at maximum speed if a FastPrep® Instrument is not available. If homogenizers from other manufacturers are used, consult the Instruction Manual or manufacturer for appropriate homogenization parameters.
4. Centrifuge at 14,000 x g for 5 mins to pellet debris.
   Note: Extending centrifugation to 10 mins can enhance elimination of excessive debris from large samples or cells with complex cell walls. Perform centrifugation at maximum speed available if 14,000 x g is not feasible.
5. Transfer the supernatant (~800 µL) to a clean 1.5 mL microcentrifuge tube. Add 250 µL **Inhibitor Removal MS** and mix by inverting the tube 20 times.

6. Centrifuge at 14,000 x g for 5 mins to pellet precipitate.
7. Transfer the supernatant (~800 µL) to a new 2.0 mL microcentrifuge tube. Add an equal volume of **Binding Buffer MS** and 5 µL **Magnetic Beads** to the supernatant. Vortex or invert the tube to mix.
   Note: Ensure Magnetic Beads are thoroughly mixed before transferring to the supernatant.
8. Place the tube on a shaker for 5 mins to allow binding.
9. Place the tube on a 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 attracting slowly, extend attraction time.
10. Add 800 µL **Wash Buffer S** to the tube and place on a shaker for 3 mins.
11. Place the tube on the magnetic rack for 1 min, allow Magnetic Beads to settle, then discard supernatant.
12. Repeat step 10 to step 11 for a second wash step.
13. Air dry Magnetic Beads for 5-10 mins at 55 ℃ by placing the tube on a heat block.
    Note: This is for removal of residual ethanol and ensure Magnetic Beads are completely dry.
14. Add 100 µL **DES Buffer** to resuspend Magnetic Beads and incubate on a heat block at 55℃ for 5 mins.
15. Place the tube on a magnetic rack for 3-5 mins until Magnetic Beads have settled, and transfer the supernatant (eluted DNA) to a clean 1.5 mL microcentrifuge tube. DNA is now ready for PCR and other downstream applications. Store at -20℃ for extended periods.
    Note: If the supernatant is too turbid or there are still Magnetic Beads remaining, please centrifuge at 14,000 x g for 3-5 mins and transfer the supernatant again.

6.2 Automated Extraction

1. Add 100 - 500 mg soil sample to a **Lysing Matrix E** tube.

   Note: MagBeads FastDNA™ Kit for Soil can extract gDNA from a wide range of soils. The amount of starting material is dependent on the biomass level in the selected soil type. Soil of high biomass, such as flowerpot soil and topsoil, are only required at 100 mg for the extraction process. After adding the sample to the tube, ensure there is still 1/3 - 1/4 empty space remaining in the tube.
2. Add 980 µL **Lysis Buffer S1**, 120 µL **Lysis Buffer S2** and 10 µL **RNase A Solution** to the sample in the Lysing Matrix E tube and vortex 5 - 10 seconds to mix.
3. Homogenize sample in a FastPrep® Instrument for 20 - 40 seconds at a speed setting of 6.0 m/s.

   Note: The speed and time can be changed according to different soil samples. Vortex 5 - 10 mins at maximum speed if a FastPrep® Instrument is not available. If homogenizers from other manufacturers are used, consult the Instruction Manual or manufacturer for appropriate homogenization parameters.
4. Centrifuge at 14,000 x g for 5 mins to pellet debris.

   Note: Extending centrifugation to 10 mins can enhance elimination of excessive debris from large samples or cells with complex cell walls. Perform centrifugation at the maximum speed available if 14,000 x g is not feasible.
5. Transfer the supernatant (~800 µL) to a clean 1.5 mL microcentrifuge tube. Add 250 µL **Inhibitor Removal MS** and mix by inverting the tube 20 times.
6. Centrifuge at 14,000 x g for 5 mins to pellet precipitate.
7. Transfer 400 µL supernatant carefully to well 2 and well 3 of a 96 - well plate. Add additional reagents into the respective wells as shown below.

8.

Well	Reagents	Volume (µL)
1	Deionized Water	800
	Magnetic Beads	5
2	Sample Supernatant	400
	Binding Buffer MS	400
3	Sample Supernatant	400
	Binding Buffer MS	400
4	Wash Buffer S	800
5	Wash Buffer S	800
6	DES Buffer	100

9. Run the instrument according to following settings.

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

10. Transfer eluted DNA from well 6 into a clean 1.5 mL microcentrifuge tube. DNA is now ready for PCR and other downstream applications. Store at -20℃ for extended periods.

    Note: If there are still Magnetic Beads remaining in eluted DNA, please centrifuge at 14,000 x g for 3 - 5 mins and transfer the supernatant again.

7. Data

MagBeads FastDNA™ Kit for Soil has been extensively tested for its performance. The following table displays gDNA yields obtained from various soil samples using the kit. Results demonstrate high yields and purity of extracted gDNA, ready-to-use for PCR amplification.

Table 1: Quality and quantity of gDNA extracted from various soil samples using MagBeads FastDNA™ Kit for Soil.

Sample	Extraction Method	Extraction Results		
		Yield (ng/mg sample)	A260/280	A260/230
Organic soil	Automation	130.25±4.95	1.84±0.00	1.23±0.02
	Manual	196.08±3.92	1.98±0.02	1.08±0.01
Flowerbed soil	Automation	19.92±0.95	1.86±0.01	1.53±0.03
	Manual	24.43±0.74	1.85±0.02	1.09±0.03
Saline soil	Automation	11.75±0.37	1.87±0.02	1.51±0.05
	Manual	13.43±0.39	1.90±0.01	1.37±0.03
Desert soil	Automation	2.47±0.13	1.85±0.00	1.43±0.03
	Manual	2.97±0.13	1.91±0.03	0.81±0.02

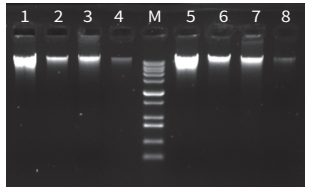

Figure1:
gDNA extracted from different soil samples using MagBeads FastDNA™ Kit for Soil, analyzed using 1% agarose gel electrophoresed at 70 V for 30 mins.
M:1kb plus DNA ladder;
Lane1-4: Automated extraction;
Lane5-8: Manual extraction;
Lane1&5: Organic soil 3 µL;
Lane2&6: Flowerbed soil 8 µL;
Lane3&7: Saline soil 8 µL;
Lane4&8: Desert soil 8 µL.

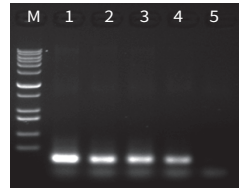

Figure 2:
PCR amplification of 16S rRNA gene from different soil samples using MagBeads FastDNA™ Kit for Soil, analyzed using 1% agarose gel electrophoresed at 70 V for 30 mins.
M: 1kb plus DNA ladder;
Lane 1: Organic soil;
Lane 2: Flowerbed soil;
Lane 3: Saline soil;
Lane 4: Desert soil;
Lane 5: Negative control.

8. Troubleshooting

8.1 Sample Handling

1. MagBeads FastDNA™ Kit for Soil is not only suitable for soil, but also for a wide range of environmental samples, including wastewater, stool, rhizosphere, gypsum, garbage, sludge, sediment, etc.
2. Wet samples: If the soil is wet, remove the water using the following protocol. First, transfer the components of Lysing Matrix E to another sterile holding tube. Then, place wet soil in the empty matrix tube and centrifuge at 14,000 x g for 30 seconds. Decant as much liquid as possible, replace Lysing Matrix E components and continue with the protocol.

8.2 Low DNA Yield

1. Ensure the extraction was performed as per the manual protocol.
2. Low microbiological content: (i) Increase amount of starting material; (ii) Process multiple samples using several Lysing Matrix tubes and then pool the samples.
3. Insufficient lysis: While a FastPrep® speed setting of 6.0 m/s and 20-40 seconds run time will be adequate for most soil types, additional processing may be necessary. If homogenizers from other manufacturers are used, consult the Instruction Manual or manufacturer for appropriate homogenization parameters. When lysing using a vortex method, the Lysing matrix tube should be well secured on the vortex through an adapter to ensure homogenization of the sample.
4. Insufficient Magnetic Beads: Beads are supplied as a suspension and must be thoroughly resuspended before aliquoting. Vigorously shake the bottle of Magnetic Beads to produce a uniform suspension; resuspension can also be performed by vortexing.

5. Magnetic Beads losing binding capacity: Ensure Magnetic Beads were stored at 2-8 ℃ upon receiving the kit; do not freeze the beads.
6. Sample loss during transfer: DNA will bind to the Magnetic Beads. Make sure all beads are attracted by the magnetic rack during transfer.
7. Isopropanol not added to Binding Buffer MS: Ensure 35 mL of isopropanol is added to the Binding Buffer MS concentrate before use.
8. Poor elution: (i) Ensure Magnetic Beads are completely resuspended with DES Buffer; (ii) Incubate the Magnetic Beads resuspended with DES Buffer for 5 mins at 55 ℃ before separation.

8.3 Low A260/280 Ratios for Purified DNA

1. Proteins not removed efficiently: Inhibitor Removal MS must be efficiently mixed in the lysate. Invert tube by hand at least 20 times or mix by pipet pumping. Incubating the sample on ice/ keeping it in the fridge for 5 mins can further precipitate proteins from difficult samples.
2. Contaminants not removed efficiently: Check that the correct volume of 100% ethanol was added to the concentrate before use. During the wash with Wash Buffer S, it is necessary to resuspend the Magnetic Beads thoroughly to efficiently remove contaminants.
3. Precipitation during washing: If precipitation occurred after addition of Wash Buffer S, disperse the precipitates by tapping the side of tube or vortex.
4. Heating after homogenization: After homogenization, do not centrifuge down the lysate - heating it at 70 ℃ for 10 mins will improve 260/280 ratios for some soil samples.

8.4 High A260/280 Ratios for Purified DNA

Possible RNA contamination, which can be confirmed when analyzed using gel electrophoresis. Add RNase A together with Lysis Buffer S1 and Lysis Buffer S2 at step 2.

8.5 Low A260/230 Ratios for Purified DNA

1. Proteins not removed efficiently: Refer to 8.3.1.
2. Contaminants not removed efficiently：Refer to 8.3.2.
3. Residual ethanol in the final eluate DNA: Increase the air-dryingtime or incubate the Magnetic Beads at 55 ℃ to speed up the drying process.

8.6 Fragmented DNA

Optimize lysing conditions: High powered bead beating cell disrupters can shear DNA if process settings are too long or powerful. While a FastPrep® speed setting of 6.0 m/s and 20-40 seconds run time will be adequate for most soil types, it is possible that lowering speed and/or duration settings will result in higher molecular weight DNA.

8.7 DNA Does Not Amplify

1. Quantify DNA yield using a spectrophotometer. High concentrations of DNA will inhibit PCR reactions.
2. Dilute DNA template: Inhibitors in the eluted DNA can inhibit PCR reactions. Dilution of template DNA can reduce such inhibition. This should not be necessary with DNA isolated with the MagBeads FastDNA™ Kit for Soil, but it 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.