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Instruction Manual

SPINeasy DNA Kit for Feces

Spin Columns for Quick Isolation of Genomic DNA from Feces

Cat. No.: 116531050 (50 Preps)
Storage: 15-30 °C

Table of Contents

1. Introduction

SPINeasy DNA Kit for Feces is a high–performance gDNA extraction kit based on spin–column technology. It enables quick isolation of gDNA from feces in less than 30 min. Briefly, fecal samples are placed into Lysing Matrix E tubes and used with FastPrep® Instruments from MP Biomedicals to effectively lyse various cells including bacteria, fungi, viruses, protists and more. A series of specially formulated reagents were developed to remove proteins and other contaminants. Column F1 (provided in the kit) has high binding capacity and selectivity for gDNA. The combined kit components provide an indispensable tool for extracting high yields of pure gDNA from fecal samples of different species. Extracted gDNA is ready for downstream analyses such as PCR, restriction digestion and sequencing. Visit www.mpbio.com to explore additional products to support your research.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysing Matrix E	50 ea	116914050
Pre–Wash Buffer	60 mL	116570402
Lysis Buffer F1	60 mL	116570403
Lysis Buffer F2	8 mL	116570404
RNase A Solution	550 µL	116530053
Inhibitor Removal FS	15 mL	116531051
Binding Buffer FS	15 mL	116531052
Wash Buffer FS1	9 mL	116531053
Wash Buffer FS2	6 mL	116531054
DES Buffer	10 mL	116530057
Column F1	50 ea	116531055
2.0 mL Collection Tubes	50 ea	116530059
1.5 mL Collection Tubes	50 ea	116530060
Instruction Manual	1 ea	–
Quick–Start Protocol	1 ea	–
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument – FastPrep–24™ 5G (Cat. No.116005500) or vortex with 2 mL tube adapter
- Microcentrifuge capable of at least 14,000 x g
- Water bath or heat block
- Absolute ethanol (71 mL)
- 2.0 mL Microcentrifuge tubes (100 pcs)

3. Storage and Stability

All SPINeasy DNA Kit for Feces components are guaranteed for at least 24 months from the date of manufacture when stored at room temperature (15–30 ℃).

4. Notes Before Starting

Please check as appropriate:

☐ Add 35 mL isopropanol to Binding Buffer FS and mark on the bottle.

☐ Add 21 mL absolute ethanol to Wash Buffer FS1 and mark on the bottle.

☐ Add 50 mL absolute ethanol to Wash Buffer FS2 and mark on the bottle.

☐ Prepare 100 x 2.0 mL microcentrifuge tubes.

☐ Lysis can be performed by vortexing the sample in a Lysing Matrix E tube at the maximum speed for 10 min 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 F1, Lysis Buffer F2, Inhibitor Removal FS, Binding Buffer FS and Wash Buffer FS1 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

A Quick–Start Protocol is provided in the kit for quick reference throughout the extraction process.

1. Add 30–200 mg feces sample to **Lysing Matrix E** tube.
   Note 1: SPINeasy DNA Kit for Feces can extract gDNA from a wide range of fecal samples. The amount of starting material is dependent on the biomass level in the selected feces type. Feces of high biomass, such human and mouse, are only required at 30 mg for the extraction process.
   Note 2: Pre–Wash Buffer is provided for additional cleaning step prior to lysis. This process is optional and can be performed by vortexing the sample in 1.0 mL Pre–Wash Buffer at full speed for 40 s, followed by discarding of the supernatant after centrifugation at 14,000 x g for 5 min.
2. Add 980 µL **Lysis Buffer F1**, 120 µL **Lysis Buffer F2** and 10 µL **RNase A Solution** to the sample; vortex to mix.
   Note: If sample is frozen, allow it to thaw at room temperature for 1–3 min after addition of lysis buffer.
3. Homogenize in a FastPrep® Instrument for 40 seconds at speed setting of 5.0 m/s.
   Note: Vortex the sample at full speed for 10 min if a FastPrep® Instrument is unavailable.
4. Centrifuge at 14,000 x g for 5 min.
   Note: Centrifuge at the maximum speed for all steps if 14,000 x g is not feasible.
5. Carefully transfer the supernatant to a clean 2.0 mL microcentrifuge tube (self–provided).
6. Add 250 µL **Inhibitor Removal FS** to the transferred supernatant and shake 10 times to mix.
7. Centrifuge at 14,000 x g for 5 min.
8. Transfer 900 µL supernatant to a clean 2.0 mL microcentrifuge tube (self–provided).
9. Add 900 µL of **Binding Solution FS** to the transferred supernatant and mix well.

10. Transfer 800 µL of the mixture to **Column F1** placed on top of a **2.0 mL Collection Tube** (provided).
11. Centrifuge at 14,000 x g for 30 s. Empty collection tube and reuse. Repeat the process once and discard the remaining mixture.
12. Add 500 µL of **Wash Buffer FS1** to the column. Centrifuge at 14,000 x g for 30 s. Empty collection tube and reuse. Repeat the washing step twice using 500 µL of **Wash Buffer FS2.**
13. Without addition of any liquid, centrifuge the empty column at 14,000 x g for 2 min to dry it.
14. Discard collection tube and place the column on top of a **1.5 mL Collection Tube** (provided).
15. Air dry the column for 5 min at room temperature.
16. Heat **DES Buffer** to 55 ℃ using a water bath while waiting.
17. Add 100 µL of pre–heated DES Buffer to the center of column.
18. Centrifuge at 14,000 x g for 1 min to elute DNA.
19. Eluted DNA is now ready for downstream applications. Store at –20 ℃ for extended periods or 4 ℃ until use.

7. Data

SPINeasy DNA Kit for Feces has been extensively tested for its performance. The following table displays gDNA yields obtained from various fecal samples using the kit. Results demonstrate high yields and purity of extracted gDNA, ready–to–use for PCR amplification of gut bacterial and host genes.

Table 1: Quality and quantity of gDNA extracted from various fecal samples using SPINeasy DNA Kit for Feces.

Sample/ mass	Extraction Results		
	Yield (ng/mg sample)	A260/280	A260/230
Bovine/ 150 mg	29.40 ± 2.87	1.89 ± 0.00	1.39 ± 0.01
Goat/ 150 mg	39.93 ± 1.41	1.96 ± 0.00	1.77 ± 0.03
Chicken/150 mg	21.65 ± 1.29	1.94 ± 0.01	1.73 ± 0.01
Swine/ 30 mg	56.00 ± 3.30	1.95 ± 0.01	1.86 ± 0.02
Mouse/ 30 mg	372.43 ± 17.57	1.98 ± 0.01	2.18 ± 0.01
Human/ 30 mg	302.10 ± 1.77	1.98 ± 0.00	2.19 ± 0.00

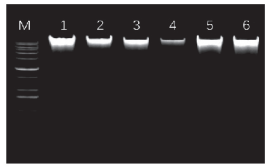

Figure 1:
gDNA extracted from different fecal samples using SPINeasy DNA Kit for Feces, analyzed using 1% agarose gel electrophoresed at 70 V for 30 min.
M: 1kb plus DNA ladder;
Lane 1: Bovine 8 µ L;
Lane 2: Goat 8 µ L;
Lane 3: Chicken 8 µ L;
Lane 4: Swine 8 µ L;
Lane 5: Mouse 4 µ L;
Lane 6: Human 4 µ L.

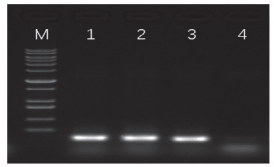

Figure 2:
PCR amplification of 16S rRNA gene from different fecal samples using SPINeasy DNA Kit for Feces, analyzed using 1% agarose gel electrophoresed at 70 V for 30 min.
M: 1kb plus DNA ladder;
Lane 1: Goat 4 µ L;
Lane 2: Mouse 4 µ L;
Lane 3: Human 4 µ L;
Lane 4: Negative control.

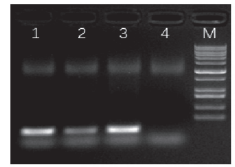

Figure 3:
PCR amplification of 12S rRNA gene from different fecal samples using SPINeasy DNA Kit for Feces, analyzed using 1% agarose gel electrophoresed at 70 V for 30 min.
M: 1kb plus DNA ladder;
Lane 1: Goat 4 µ L;
Lane 2: Mouse 4 µ L;
Lane 3: Human 4 µ L;
Lane 4: Negative control.

8. Troubleshooting

8.1 Sample Handling

1. Sample type: SPINeasy DNA Kit for Feces is suitable for a wide range of fecal samples including intestinal content.
2. Wet samples: If the sample is very diluted, remove water by using the following protocol. First, centrifuge the diluted sample at 14,000 x g for 10 min. Decant as much liquid as possible. Resuspend the sample in lysis buffers, transfer the suspension to Lysing Matrix E tube and continue with the extraction protocol.
3. Highly contaminated sample: Remove contaminants prior to lysis by using Pre–Wash Buffer. Vortex the sample in 1.0 mL Pre–Wash Buffer at full speed for 40 s, followed by discarding of the supernatant after centrifugation at 14,000 x g for 5 min. Processed sample is then extracted according to the protocol.

8.2 Low DNA Yields

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 5.0 m/s and 40 seconds run time will be adequate for most fecal samples, additional processing may be necessary. Homogenization strength can be increased to 6.0m/s or lysis time prolonged to 60–80 seconds. 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. Increase DNA capture: Instead of transferring 2 x 800 µL of DNA–Binding Buffer FS mixture to the column, transfer the entire volume.
5. Poor elution: (i) Ensure the DES Buffer is heated to 55 ℃ and is loaded to the center of the column during elution; (ii) Incubate the column with added DES Buffer for 5 min at 55 ℃ prior to elution.

8.3 Low A260/A280 Ratios for Purified DNA

1. Proteins not removed efficiently: Inhibitor Removal FS must be efficiently mixed in the lysate. Invert tube by hand at least 10 times or mix by pipet pumping. Incubating the sample on ice/ keeping it in the fridge for 5 min can help to further precipitate proteins from difficult samples.
2. Contaminants not removed efficiently: (i) Remove contaminants prior to lysis by using Pre–Wash Buffer; refer to 8.1.3. (ii) Washing should be carried out twice using Wash Buffer FS2.

8.4 High A260/A280 Ratios for Purified DNA

Possible RNA contamination, which can be confirmed via gel electrophoresis analysis. Incubate sample with RNase A Solution

for 5 min after the lysis step before spinning down the debris.

8.5 Low A260/A230 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 eluted DNA: (i) Increase centrifugation speed or time to dry spin the column, (ii) Increase the air-drying time of Column F1 or (iii) Incubate the column in a 55 ℃ oven to speed up the drying process.

8.6 Low DNA Quality

1. Optimize lysis conditions: High powered bead beating cell disrupters can shear DNA if process settings are too long or powerful. While a FastPrep® speed setting of 5.0 m/s and 40 seconds run time will be adequate for most fecal samples, it is possible that lowering the speed and/or duration settings will result in higher molecular weight DNA.
2. Equal volume of binding buffer: Supernatant from step 8 may not be exactly 900 µL after centrifugation. Add equal volume of Binding Buffer FS to transferred supernatant at step 9.
3. Pre-wash: Treat the sample according to 8.1.3.

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 SPINeasy DNA 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 a more aggressive lysing matrix (such as Lysing Matrix A, Cat. No.116910050) 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.