For Research Use Only

INSTRUCTION MANUAL

Fecal DNA Extraction Kit

IB47820 (4 Preparation Sample Kit)

IB47821 (50 Preparation Kit)

IB47822 (100 Preparation Kit)

Advantages

Sample: 180-200 mg of fresh or frozen stool samples
Binding capacity: 50 µg
Format: beadbeating tubes, proprietary inhibitor removal buer, PCR inhibitor removal columns and genomic DNA spin columns
Time: within 50 minutes
Elution Volume: 30-100 µl
Kit Storage: dry at room temperature (15-25°C)

Introduction

The Fecal DNA Extraction Kit is designed for rapid isolation of genomic DNA from microorganisms, such as bacteria and fungi in stool samples. The stool sample is homogenized and disrupted using a lysis buffer and ceramic beads. Insoluble particles, proteins and PCR inhibitors such as humic acid are then precipitated with a proprietary inhibitor removal buffer. Residual inhibitors are further removed by passing through a specialized PCR inhibitor removal column. Genomic DNA in the sample is then bound by the GD Column followed by wash and elution. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 50 minutes. The purified genomic DNA is ready for use in PCR, restriction enzyme digestion, and sequencing reactions.

Quality Control

The quality of the Fecal DNA Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 mg stool samples. Following the purification process, the purified genomic DNA (A260/A280 ratio between 1.7-2.0) is analyzed by electrophoresis.

Kit Contents

Component	IB47820	IB47821	IB47822
ST1 Buffer[1]	2 ml x 2	50 ml	85 ml
ST2 Buffer	1 ml	15 ml	30 ml
ST3 Buffer	10 ml	45 ml x 2	160 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)
Elution Buffer	1 ml	6 ml	30 ml
Inhibitior Removal Column	4 pcs	50 pcs	100 pcs
GD Column	4 pcs	50 pcs	100 pcs
Beadbeating Tube (Type C)	4 pcs	50 pcs	100 pcs
2 ml Centrifuge Tube	4 pcs	50 pcs	100 pcs
2 ml Collection Tube	4 pcs	50 pcs	100 pcs

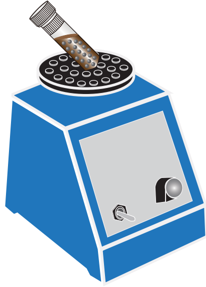
[1] If precipitates have formed in ST1 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

[2] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

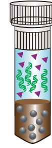
IMPORTANT BEFORE USE!

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

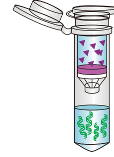
Quick Protocol Diagram



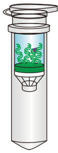
1. Stool sample homogenization and lysis with Beadbeating Tube and ST1 Buffer



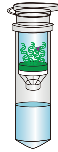
2. PCR inhibitor removal preparation using ST2 Buffer



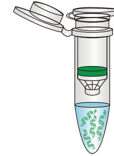
3. PCR inhibitor removal using the Inhibitor Removal Column



4. DNA binding to membrane while contaminants remain suspended



5. Wash (removal of contaminants while DNA remains bound to membrane)



6. Elution of pure genomic DNA which is ready for subsequent reactions

Fecal DNA Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

If precipitates have formed in ST1 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:
1.5 ml microcentrifuge tubes, standard vortex, absolute ethanol.

Protocol Procedure

1. Sample Lysis

Transfer 180-220 mg of stool to a Beadbeating Tube containing ceramic beads.

NOTE: For very dry or fiber rich animal stool samples, such as sheep, rabbit or mouse feces, reduce the stool amount to 60-80 mg, as the stool sample will soak up the lysis buffer. Human stool samples may contain undigested food, such as crop or fruit husks. These particles should not be transferred.

Add 800 μl of ST1 Buffer. Vortex briefly then incubate at 70°C for 5 minutes. Attach the Beadbeating Tubes horizontally to a standard vortex by taping or using an adapter. Vortex at maximum speed for 10 minutes at room temperature. Centrifuge the Beadbeating Tubes at 8,000 x g for 2 minutes at room temperature to eliminate the foam caused by detergents present in ST1 Buffer. Transfer 500 μl of supernatant to a new 1.5 ml microcentrifuge tube.
NOTE: Preheat the required Elution Buffer (100 μl per sample) to 60°C for DNA elution.

2. PCR Inhibitor Removal
Add 150 μl of ST2 Buffer then vortex for 5 seconds. Incubate at 0-4°C for 5 minutes. Centrifuge at 16,000 x g for 3 minutes at room temperature to precipitate insoluble particles and PCR inhibitors. Place an Inhibitor Removal Column (purple ring) in a 2 ml Centrifuge Tube. Transfer 500 μl of clear supernatant to the Inhibitor Removal Column. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the column. Save the flow-through in the 2 ml Centrifuge Tube for DNA Binding.
NOTE: If a pellet is in the flow-through, transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (not provided).

3. DNA Binding
Add 800 μl of ST3 Buffer to the flow-through then mix IMMEDIATELY by shaking vigorously for 5 seconds. Place a GD Column (green ring) in a 2 ml Collection Tube. Transfer 700 μl of sample mixture to the GD Column. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. Transfer the remaining sample mixture to the GD Column. Centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.

4. Wash
Add 400 μl of ST3 Buffer to the GD Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Add 600 μl of Wash Buffer (make sure absolute ethanol was added) to the GD Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Add 600 μl of Wash Buffer (make sure absolute ethanol was added) to the GD Column again. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

5. Elution
Transfer the dry GD Column to a new 1.5 ml microcentrifuge tube. Add 30-100 μl of preheated Elution Buffer[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1] If a higher DNA concentration is required, use 30 μl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 μl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 μl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.
[2] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.
[3] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH2O should be fresh as ambient CO2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting

Low Yield

Too much starting material.
Too much stool was added to the Beadbeating Tube. An ample amount of space is required in the Beadbeating Tube to allow the beads to efficiently disrupt the sample.

Sample lysis or homogenization was incomplete.
Horizontally vortex the Beadbeating Tube at the maximum speed using a vortex at room temperature for 10 minutes or using a Disruptor Genie or similar.

Incorrect DNA elution.
Pre-heat the Elution Buffer to 60°C prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely.

Inappropriate buffer preparation.
Add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use. If precipitates have formed in ST1 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

Sample material not stored properly.
Stool samples should be kept at 2-8°C for only a few hours after collection or frozen at -20°C until DNA extraction. Frozen stool samples should be thawed on ice immediately before extraction.

Degraded DNA

Mechanical sample disruption is too vigorous.
Reduce intensity or incubation time of mechanical sample lysis.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.
Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

PCR inhibitor contamination.
Use diluted DNA (1:10) as a template to reduce the concentration of PCR inhibitors for PCR reactions. DNA can be further purified using the GenepHlowTM PCR Cleanup Kit to eliminate PCR inhibitors.

Fecal DNA Extraction Kit Functional Test Data

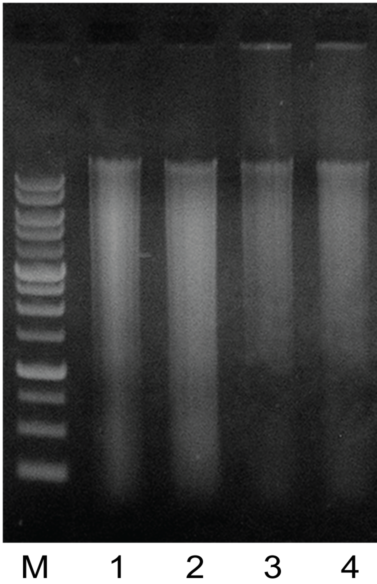


Figure 1.
Total DNA was purified from 150 mg of human stool samples which were frozen at -20°C for 1 week and from 80 mg of rabbit stool samples which were stored at room temperature for 1 week using the Fecal DNA Extraction Kit. 5 µl aliquots of purified DNA from 100 µl eluates were analyzed on a 0.8% agarose gel.
M = 1 Kb DNA Ladder
Lanes 1-2 = Human stool samples
Lanes 3-4 = Rabbit stool samples

	ng/ul	260/280	260/230	Yield (µg)
1	127.1	1.86	2.12	12.7
2	129.9	1.87	2.22	13.0
3	77.6	1.85	2.15	7.7
4	82.6	1.85	2.15	8.3

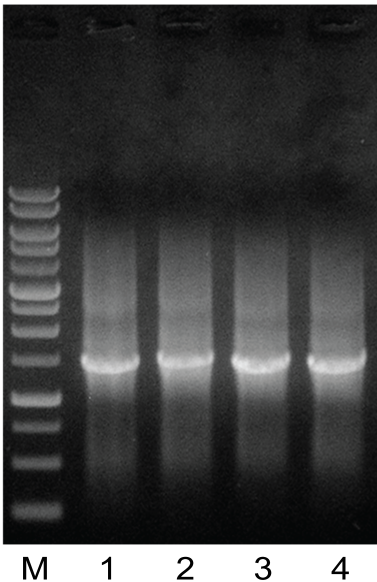


Figure 2.
5 µl of undiluted, purified DNA from human and rabbit stool, served as a template for amplification of a 1.5 kb fragment from the bacterial 16S rRNA gene. The extracted DNA works undiluted in a PCR reaction, indicating the successful removal of PCR inhibitors.
M = 1 Kb DNA Ladder
Lanes 1-2 = Human stool samples
Lanes 3-4 = Rabbit stool samples

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