



ISOLATE DNA Kit

Product Manual

ISOLATE Fecal DNA Kit



A Meridian Life Science® Company

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ISOLATE Fecal DNA Kit

1. KIT CONTENTS

REAGENT	50 PREPS	100 PREPS
Lysis Buffer	40ml	2 x 40ml
DNA Pre-Wash Buffer	15ml	2 x 15ml
Fecal DNA Wash Buffer	50ml	2 x 50ml
Fecal DNA Binding Buffer	100ml	2 x 100ml
DNA Elution Buffer	10ml	2 x 10ml
Bashing Beads Lysis Tubes	50	2 x 50
Spin Filters (orange cap)	50	2 x 50
Spin Filters (green cap)	50	2 x 50
Spin Columns	50	2 x 50
Collection Tubes	200	2 x 200
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2. DESCRIPTION

The isolation of DNA from feces can be challenging. ISOLATE Fecal DNA Kit is specifically developed for the simple, rapid isolation of high quality DNA from a variety of fecal samples including humans, birds, rats, mice, cattle, etc. Bacterial, protist, as well as host DNA can be effectively isolated from ≤ 150 mg sample of mammalian feces. The procedure is easy and can be completed in as little as 15 minutes. Fecal samples are added directly to a Bashing Beads Lysis Tube and rapidly lysed by bead beating in a vortex, without the use of organic denaturants or proteinases. The DNA is then bound, isolated and purified using spin columns. The eluted DNA, free of contaminants and enzyme inhibitors, is ideal for downstream molecular biology applications including PCR, arrays, genotyping, etc.

Features

- 15-minute isolation protocol
- High quality PCR-Ready DNA
- Suitable for a variety of fecal samples
- No need for organic denaturants or proteinases

Applications

Isolation of DNA from:

- Humans
- Birds
- Rats, mice
- Cattle

3. STORAGE

The ISOLATE Fecal DNA Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS
Starting material

Up to 150mg fecal samples from

- Humans
- Birds
- Rats, mice
- Cattle

DNA Purity

Typical A_{260}/A_{280} ratio > 1.8

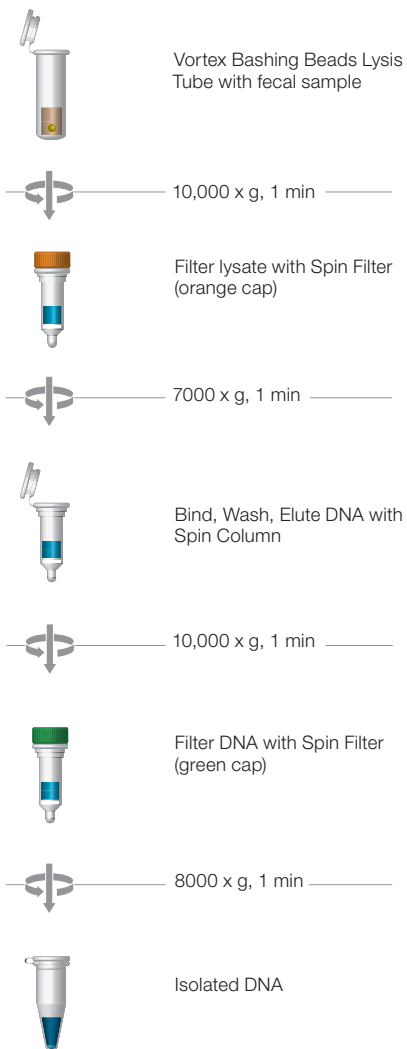
DNA recovery

Up to 25 μ g total DNA is eluted into 100 μ l
 For DNA 75bp to 10kb, recovery is 70-90%.
 For DNA 11kb to 23kb, recovery is 50-70%.

Time required

15 minutes

Isolation of fecal DNA



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

- Microcentrifuge
- Vortex
- Cell disrupter (optional)
- β -mercaptoethanol

7. PROTOCOL

7.1 Isolation of fecal DNA

For optimal performance, add β -mercaptoethanol (user supplied) to the Fecal DNA Binding Buffer to a final dilution of 0.5% i.e., 500 μ l per 100ml).

Before you start:

- The supplied Spin Filters (green caps) need to be prepared prior to use as follows:
 - i. Snap off the base
 - ii. Insert into a Collection Tube
 - iii. Spin in a microcentrifuge at exactly 8,000 x g for 3 minutes.

Note: If the spin filter matrix is dry, add 400-600 μ l water prior to prepping the filter.
- To prepare Spin Filter (orange cap), snap off the base before use.
- If a precipitate has formed in the DNA Pre-Wash Buffer, re-suspend by incubating the bottle at 30-37°C for 30 minutes and mix by inversion. Do not microwave.

1. Add up to 150mg of fecal sample to a Bashing Bead Lysis Tube. Add 750 μ l Lysis Buffer to the tube. Cap tube tightly to prevent leakage.
2. Secure in a bead beater fitted with a 2ml tube holder assembly and process at maximum speed for 5 minutes.

Note: Processing times may be as little as 40 seconds when using high-speed cell disrupters. Alternatively, a standard bench-top vortex may be used, although the overall DNA yield may be lower.
3. Centrifuge the Bashing Bead Lysis Tube in a microcentrifuge at $\geq 10,000$ x g for 1 minute.
4. Transfer up to 400 μ l supernatant to a Spin Filter (orange top) placed in a Collection Tube and centrifuge at 7000 x g for 1 minute.

Note: Ensure that the base of the Spin Filter (orange top) has been snapped off.
5. Add 1,200 μ l of Fecal DNA Binding Buffer to the filtrate in the Collection Tube from Step 4.
6. Transfer 800 μ l of the mixture from Step 5 to a Spin Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.

Note: The Spin Column has a maximum capacity of 800 μ l.

7. Discard the flow through from the Collection Tube and repeat Step 6 with the remaining mixture from Step 5.
8. Add 200µl DNA Pre-Wash Buffer to the Spin Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute.
9. Add 500µl Fecal DNA Wash Buffer to the Spin Column and centrifuge at 10,000 x g for 1 minute.
10. Transfer the Spin Column to a clean 1.5ml microcentrifuge tube. Add 100µl (25µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
11. Transfer the eluted DNA from Step 10 to a prepared Spin Filter (green top) in a clean 1.5ml microcentrifuge tube and centrifuge at exactly 8,000 x g for 1 minute.
Note: Ensure that the Spin Filter (green top) has already been prepared as per instructions.
12. The isolated DNA is suitable for use in downstream applications.

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Low DNA yield	Insufficient homogenization of sample	Repeat protocol using new sample and ensure complete homogenization.
	Incomplete elution	Incubate sample in elution tube with RNase-free water for up to 5 minutes and repeat elution step.
A_{260}/A_{280} ratio too high	RNA contamination	Add 20µl of RNase A (20mg/ml) to the eluate and incubate for 10 minutes at room temperature.
A_{260}/A_{280} ratio too low	Protocol not followed correctly	Repeat purification with new sample.
DNA does not perform well in downstream applications	Contaminants, enzyme inhibitors not removed	Repeat purification with new sample.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please call us on:

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US: +1 508 880 8990

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or email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Fecal DNA Kit	50 Preps	BIO-52082
	100 Preps	BIO-52038

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Plasmid Mini Kit	50 Reactions	BIO-52056
ISOLATE II Genomic DNA Kit	50 Reactions	BIO-52066
ISOLATE II Plant DNA Kit	50 Reactions	BIO-52069
ISOLATE II PCR and Gel Kit	50 Reactions	BIO-52058
RiboSafe RNase Inhibitor	2500 Units	BIO-65027
Proteinase K	100mg	BIO-37037
PCR Water	10 x 10ml	BIO-37080
Agarose	500g	BIO-41025

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.



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