DATA SHEET

ISOLATE DNA Kit

Product Manual

ISOLATE Fecal DNA Kit

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ISOLATE Fecal DNA Kit

1. KIT CONTENTS

REAGENT	25 REACTIONS	100 REACTIONS
Lysis Buffer	20ml	2 x 40ml
DNA Pre-Wash Buffer	7.5ml	2 x 15ml
Fecal DNA Wash Buffer	25ml	2 x 50ml
Fecal DNA Binding Buffer	50ml	2 x 100ml
DNA Elution Buffer	5ml	2 x 10ml
Bashing Beads Lysis Tubes	25	2 x 50
Spin Filters (orange cap)	25	2 x 50
Spin Filters (green cap)	25	2 x 50
Spin Columns	25	2 x 50
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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 ≤150mg 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 RNA 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

Time required

15 minutes

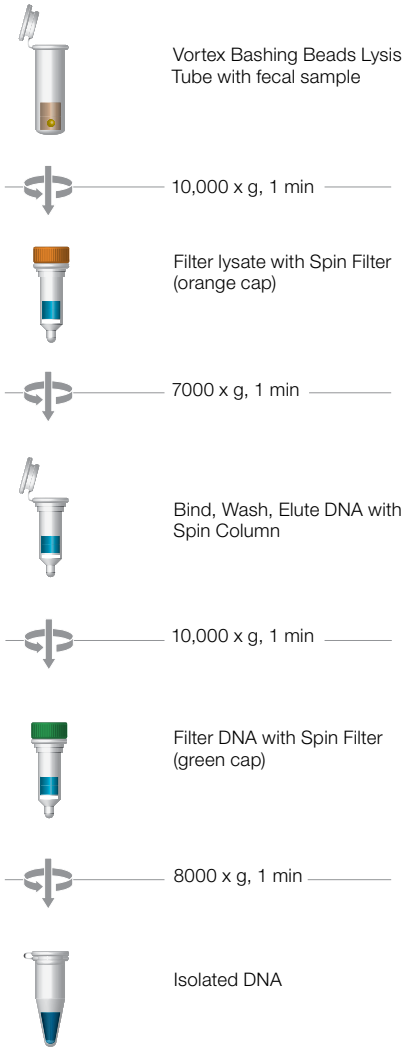
DNA Purity

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

DNA recovery

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

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., 500µl 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 400-600µl 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 750µl 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 ≥10,000 x g for 1 minute.**

4. **Transfer up to 400µl 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 1,200µl of Fecal DNA Binding Buffer to the filtrate in the Collection Tube from Step 4.**

6. **Transfer 800µl 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 800µl.

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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B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Fecal DNA Kit	25 Reactions	BIO-52037
ISOLATE Fecal DNA Kit	100 Reactions	BIO-52038

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Plasmid Mini Kit	50 Reactions	BIO-52026
ISOLATE Genomic DNA Mini Kit	50 Reactions	BIO-52032
ISOLATE Plant DNA Mini Kit	50 Reactions	BIO-52035
ISOLATE PCR and Gel Kit	50 Reactions	BIO-52029
RiboSafe RNase Inhibitor	2500 Units	BIO-65027
Proteinase K	100mg	BIO-37037
PCR Water	10 x 10ml	BIO-37080
Agarose	500g	BIO-41025
50x TAE Buffer	5 Pouches	BIO-37103
10x TBE Buffer	10 Pouches	BIO-37104

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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