



# ISOLATE II

## Fecal DNA Kit

### Product Manual



## ISOLATE II Fecal DNA Kit

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# 1. KIT CONTENTS

<b>REAGENT</b>	<b>50 Preps</b>	<b>100 Preps</b>
Lysis Buffer	40 mL	2 x 40 mL
DNA Pre-Wash Buffer	15 mL	2 x 15 mL
Fecal DNA Wash Buffer	50 mL	2 x 50 mL
Fecal DNA Binding Buffer	100 mL	2 x 100 mL
DNA Elution Buffer	10 mL	2 x 10 mL
Fecal Prep Solution	30 mL	2 x 30 mL
Bashing Beads Lysis Tubes	50	2 x 50
Spin II A Filters	50	2 x 50
Spin II B Filters	50	2 x 50
Spin Columns	50	2 x 50
Collection Tubes	200	2 x 200
Bench Protocol Sheet	1	1

## 2. DESCRIPTION

The isolation of DNA from feces can be challenging. ISOLATE II Fecal DNA Kit is specifically developed for the simple rapid isolation of inhibitor-free, high-quality DNA from a variety of fecal (including humans, birds, rats, mice, cattle, etc.) and soil (including clay, sandy, silty, peaty, chalky and loamy soils) samples. ISOLATE II Fecal DNA Kit can be used to successfully isolate DNA from difficult to lyse Gram-positive and Gram-negative bacteria, fungi, algae etc., that inhabit fecal and soil samples. The procedure is easy and can be completed in as little as 15 minutes. Fecal samples ( $\leq 150$  mg each) or soil samples ( $\leq 250$  mg each) are added directly to a Bashing Beads Lysis Tube (0.1 & 0.5 mm) and rapidly and efficiently lysed by bead beating in a vortex, without the use of organic denaturants or proteinases. Spin II technology is then used to isolate DNA which is subsequently filtered to remove humic acids/polyphenols that inhibit PCR. The eluted DNA is ideal for downstream molecular-based applications including PCR, arrays, genotyping, etc.

### Features

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

### Applications

Isolation of DNA from:

- Humans
- Birds
- Rats, mice
- Cattle

## 3. STORAGE

The ISOLATE II 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](http://www.bioline.com).

## 5. PRODUCT SPECIFICATIONS

### Starting material

Up to 150 mg fecal samples from

- Humans
- Birds
- Rats, mice
- Cattle

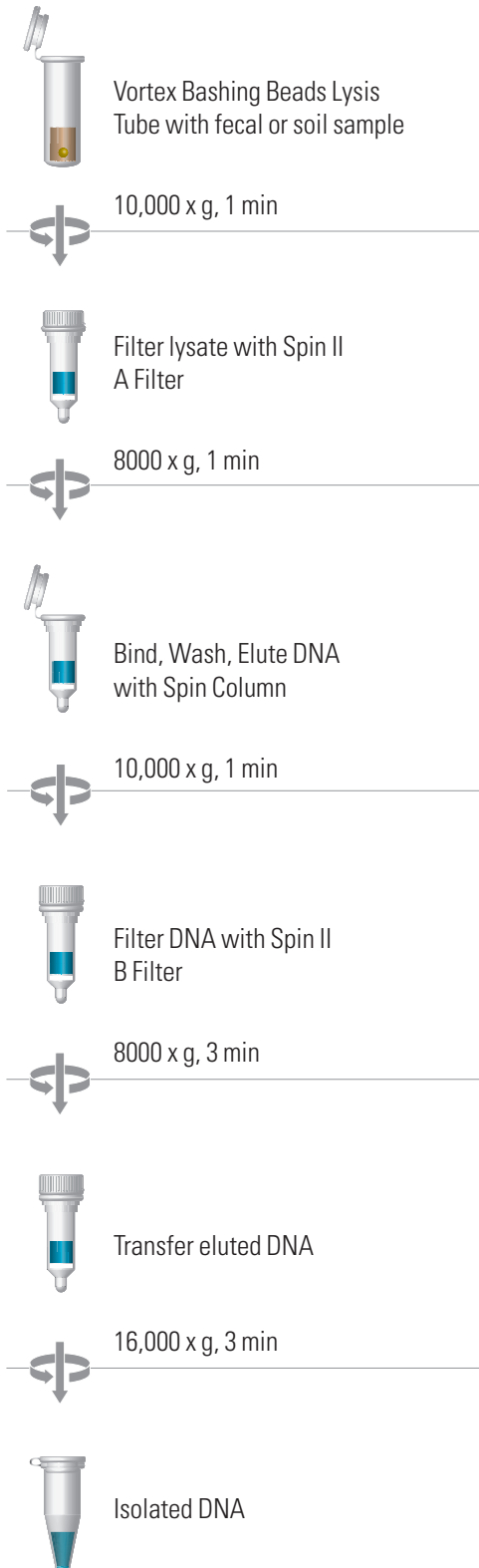
Or up to 250 mg of soil

**Time required:** 15 minutes

**DNA Purity:** Typical  $A_{260}/A_{280}$  ratio > 1.8

**DNA recovery:** Up to 25 µg total DNA is eluted into 100 µL (50 µL minimum)

## Isolation of fecal DNA



## 6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- Microcentrifuge
- Vortex
- Cell disrupter (optional)
- $\beta$ -mercaptoethanol

## 7. PROTOCOL

### 7.1 ISOLATION OF FECAL DNA

For optimal performance, add  $\beta$ -mercaptoethanol (user supplied) to the Fecal DNA Binding Buffer to a final dilution of 0.5%, i.e. 500  $\mu$ L per 100 mL.

Before you start:

- 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  $\leq$  150 mg of fecal sample or  $\leq$  250 mg of soil sample to a Bashing Bead Lysis Tube. Add 750  $\mu$ L Lysis Buffer to the tube. Cap tube tightly to prevent leakage.
2. Secure in a bead beater fitted with a 2 mL 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  $\mu$ L supernatant to a Spin II A Filter placed in a Collection Tube and centrifuge at 8000 x g for 1 minute.
5. Add 1,200  $\mu$ L of Fecal DNA Binding Buffer to the filtrate in the Collection Tube from Step 4.
6. Transfer 800  $\mu$ 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  $\mu$ L.

7. Discard the flow through from the Collection Tube and repeat Step 6 with the remaining mixture from Step 5.
8. Add 200  $\mu$ L 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 500  $\mu$ L 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 1.5 mL microcentrifuge tube. Add 100  $\mu$ L (50  $\mu$ L minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
11. Place Spin II B Filter in a clean Collection Tube and add 600  $\mu$ L Fecal Prep Solution. Centrifuge at 8000 x g for 3 minutes.
12. Transfer the eluted DNA to a prepared Spin II B Filter in a clean 1.5 mL microcentrifuge tube and centrifuge at exactly 16,000 x g for 3 minutes.
13. The isolated DNA is suitable for use in downstream applications.



## 8. TROUBLESHOOTING GUIDE

### LOW DNA YIELD

POSSIBLE CAUSE	RECOMMENDED SOLUTION
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

POSSIBLE CAUSE	RECOMMENDED SOLUTION
RNA contamination	Add 20 $\mu$ L of RNase A (20 mg/mL) to the eluate and incubate for 10 minutes at room temperature.

### $A_{260}/A_{280}$ RATIO TOO LOW

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Protocol not followed correctly	Repeat purification with new sample.

### DNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Contaminants, enzyme inhibitors not removed	Repeat purification with new sample.

## A. TECHNICAL SUPPORT

For technical assistance or more information on this product, please email us at [mbi.tech@meridianlifescience.com](mailto:mbi.tech@meridianlifescience.com)

## B. ORDERING INFORMATION

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

## C. ASSOCIATED PRODUCTS

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

## D. PRODUCT WARRANTY AND DISCLAIMER

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



### Technical Support

For technical assistance or more information on these products, please contact us at [mbi.tech@meridianlifescience.com](mailto:mbi.tech@meridianlifescience.com) or call us on +49 (0) 3371 60222 03

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