



# **ISOLATE II** Fecal DNA Kit

Product Manual





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## ISOLATE II Fecal DNA Kit

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### 1. KIT CONTENTS

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REAGENT	50 PREPS	100 PREPS
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

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Vortex Bashing Beads Lysis  
Tube with fecal or soil sample



10,000 x g, 1 min



Filter lysate with Spin II A  
Filter



8000 x g, 1 min



Bind, Wash, Elute DNA with  
Spin Column



10,000 x g, 1 min



Filter DNA with Spin II B Filter



8000 x g, 3 min



Transfer eluted DNA



16,000 x g, 3 min



Isolated DNA



## 6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE 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  $\geq 10,000 \times g$  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  $\times g$  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  $\times g$  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  $\times g$  for 1 minute.

9. Add 500  $\mu\text{L}$  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  $\mu\text{L}$  (50  $\mu\text{L}$  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  $\mu\text{L}$  Fecal Prep Solution. Centrifuge at 8000 x g for 3 minutes.
12. Transfer the eluted DNA from Step 11 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

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Low DNA yield	Insufficient homogenization of sample Incomplete elution	Repeat protocol using new sample and ensure complete homogenization. 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 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	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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or email us at [tech@bioline.com](mailto:tech@bioline.com)

### B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II 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 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

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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