



<b>Product Insert</b>
<b>Fecal PCR Kit</b>

**Catalogue Numbers:**

BIO-21100                    100 Reactions  
 BIO-21097                    25 Reactions

**Features**

- From fecal matter to PCR amplification
- Avoids the need for mouse-tail clippings or other invasive procedures to extract genomic DNA
- Contains ImmoMix™ for quick and easy amplification

**Applications**

- Downstream applications requiring high purity amplified DNA

**Description**

The Fecal PCR Kit contains all the necessary components to extract DNA from your fecal sample, and subsequently perform PCR assays on this DNA template.

The Kit has been validated on fecal samples of mouse, rat, rabbit and human origin. Bacterial, protist, as well as host DNA can be effectively isolated from a ≤150 mg sample of mammalian feces.

**Kit components:**

Box	Component	25 Reactions	100 Reactions
1 of 2	Fecal DNA Wash Buffer	25ml	100ml
1 of 2	Bashing Bead Lysis Tubes	25	100
1 of 2	Fecal DNA Binding Buffer	50ml	2 x 100ml
1 of 2	DNA Pre-Wash Buffer*	7.5ml	30ml
1 of 2	DNA Elution Buffer	5ml	20ml
1 of 2	Spin Filters (Orange Caps)	25	100
1 of 2	Spin Filters (Green Caps)	25	100
1 of 2	Spin Columns	25	100
1 of 2	Collection Tubes	2 x 50	8 x 50
2 of 2	ImmoMix (Cat No: BIO-25019)	625µl	2 x 1.25ml

**Product Specifications**

**Batch details:**

Batch No:                    See vial  
 Units per vial:            See vial  
 Concentration:            See vial

**Important**

**Storage Conditions:**

The DNA extraction components can be stored for 6 months at room temperature. ImmoMix can be stored for 6 months at -20°C.

**Shipping Conditions:**

ImmoMix on Dry Ice or Blue Ice. DNA extraction components shipped at ambient temperature.

**Associated Products:**

Product Name	Pack Size	Cat No
HyperLadder IV	200 Lanes	BIO-33029
Agarose	100g	BIO-41026
SureClean Plus	1 x 5ml	BIO-37047
dNTP Mix	500µl	BIO-39028

**Notes**

1. ImmoMix is a Trademark of Bioline.
2. This product insert is a declaration of analysis at the time of manufacture.
3. Research Use Only.

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**See Overleaf for Reaction Conditions and Recommendation**

\* A precipitate may form in the DNA Pre-Wash Buffer. To resuspend the buffer, incubate the tube at 30-37°C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

## **Protocol**

### **A. DNA Extraction:**

**Preparation prior to experiment:** Supplied Spin Filters (green caps) need to be prepared prior to use by: 1) snapping off the base, 2), inserting into a Collection Tube, and 3), spinning in a microcentrifuge at exactly 8,000 x g for 3 minutes.

1. Add up to 150mg of fecal sample to a **BashingBead Lysis Tube**.
2. Secure in a bead beater fitted with a 2.0ml tube holder assembly and process at maximum speed for 5 minutes. Alternatively, if equipment is not available, vortex sample vigorously for 5 minutes.
3. Centrifuge the **BashingBead 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 Filter** (orange cap) in a **Collection Tube** and centrifuge at 7,000rpm ( $\sim 7,000 \times g$ ) for 1 minute.
5. Add 1.2ml 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.
7. Discard the flow through from the **Collection Tube** and repeat Step 6.
8. Add 200 $\mu$ l **DNA Pre-Wash Buffer** to the **Spin Column** in a new **Collection Tube (or standard 1.5ml microcentrifuge tube)** and centrifuge at  $10,000 \times g$  for 1 minute.
9. Add 500 $\mu$ l **Fecal DNA Wash Buffer** to the **Spin Column** and centrifuge at  $10,000 \times g$  for 1 minute.
10. Transfer the **Spin Column** to a clean 1.5ml microcentrifuge tube and add 100 $\mu$ l **DNA Elution Buffer** directly to the column matrix. Centrifuge at  $10,000 \times g$  for 30 seconds to elute the DNA.
11. Transfer the eluted DNA from Step 10 to a prepared **Spin Filter** (green cap) (see preparation instructions above) in a clean 1.5ml microcentrifuge tube and centrifuge at exactly  $8,000 \times g$  for 1 minute. The filtered DNA is now suitable for PCR and other downstream applications.

### **B. DNA Amplification:**

Bioline ImmoMix is designed with ease-of-use in mind. Each reaction requires 25 $\mu$ l of 2x ImmoMix (supplied) in addition to primers and template, and 18.2m $\Omega$  water for a final reaction volume of 50 $\mu$ l.

#### **Reaction Conditions for a 50 $\mu$ l PCR**

Component	Amount
Fecal DNA template	2-5 $\mu$ l
2 x Immomix	25 $\mu$ l
Forward primer	200-900nM
Reverse primer	200-900nM
Nuclease-free dH <sub>2</sub> O	Up to 50 $\mu$ l

**Program a thermal cycler for the following PCR conditions:**

Temperature	Duration	Cycles
95 °C	10 minutes	1
94 °C	30 sec	30-40
Annealing Temperature	30 sec	
72 °C	15-30 sec/kb	
72 °C	10 minutes	1

Low template concentrations may result in smearing. This can be remedied by reducing the duration of the 95°C activation step.

An additional tube of 50mM MgCl<sub>2</sub> is provided should any fine adjustments be necessary. The table below shows the volume of MgCl<sub>2</sub> that must be added to a 50 $\mu$ l final reaction to achieve the desired final concentration.

Final MgCl <sub>2</sub> Required	MgCl <sub>2</sub> to be added
1.5mM	0 $\mu$ l
2.0mM	0.5 $\mu$ l
2.5mM	1 $\mu$ l

**This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.**