Fecal PCR Kit

DATA SHEET

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
SureCleam Plus	1 x 5ml	BIO-37047
dNTP Mix	500µl	BIO-39028

Notes
Research Use Only.

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 ≥10,000 x *g* for 1 minute.
4. Transfer up to 400µl supernatant to a **Spin Filter** (orange cap) in a **Collection Tube** and centrifuge at 7,000rpm (~7,000 x *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 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.
7. Discard the flow through from the **Collection Tube** and repeat Step 6.
8. Add 200µl **DNA Pre-Wash Buffer** to the **Spin Column** in a new **Collection Tube (or standard 1.5ml microcentrifuge 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 and add 100µl **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 cap) (see preparation instructions above) in a clean 1.5ml microcentrifuge tube and centrifuge at exactly 8,000 x *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 25µl of 2x ImmoMix (supplied) in addition to primers and template, and 18.2mΩ water for a final reaction volume of 50µl.

Reaction Conditions for a 50µl PCR

Component	Amount
Fecal DNA template	2-5µl
2 x Immomix	25µl
Forward primer	200-900nM
Reverse primer	200-900nM
Nuclease-free dH_2O	Up to 50µl

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 $MgCl_2$ is provided should any fine adjustments be necessary. The table below shows the volume of $MgCl_2$ that must be added to a 50µl final reaction to achieve the desired final concentration.

Final $MgCl_2$ Required	$MgCl_2$ to be added
1.5mM	0µl
2.0mM	0.5µl
2.5mM	1µl

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