Fecal DNA Extraction Kit

ISOFECAL for Beads Beating Manual (First edition)

Code No. 315-06281

NIPPON GENE CO., LTD.

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I Product description

ISOFECAL for Beads Beating is a kit for extracting DNA from the fecal samples.
ISOFECAL for Beads Beating uses both chemical lysis by a surface-active agent and physical disruption of cells by beads beating as the DNA extraction method. As a result, DNA can be extracted from microorganisms having strong cell walls, and fecal DNA reflecting actual fecal microflora can be obtained.
However, users should be aware that DNA extracted by ISOFECAL for Beads Beating may have been subjected to physical shearing from beads beating.

II Contents of kit

Beads Tubes	50 Tubes	
Lysis Solution F*	50 ml	× 1
Purification Solution*	20 ml	× 1
Precipitation Solution	40 ml	× 1
Wash Solution	50 ml	× 1
TE (pH8.0)	5 ml	× 1
Ethachinmate	100 µl	× 1
Manual		× 1

*: White crystal deposition may take place in the Lysis Solution F and the Purification Solution, but this will not affect quality or performance. In such cases, use after completely dissolving the crystals by incubating the whole container at about 37-65°C (mix occasionally).

III Storage

All the reagents included in ISOFECAL for Beads Beating can be stored at room temperature. However, for the Precipitation Solution, the Wash Solution and the Ethachinmate, we recommend that care be taken to prevent contamination at the time of use (contamination by fungi and bacteria), with storage at a low temperature (2-10°C) after opening.

IV Precautions

- This product is a reagent for research and cannot be used for medical or other objectives.
- This product should be handled only by persons having basic knowledge of reagents.
- Handle this product in accordance with the descriptions in the manual.
- We are not responsible for problems caused if this product is not handled in accordance with the manual.
- A patent has been filed for fecal DNA extraction with ISOFECAL for Beads Beating by the University of Tokyo TLO. Nippon Gene has been licensed to practice the fecal DNA extraction method by the University of Tokyo TLO.

V Protocol

< Reagents, instruments, etc., required in addition to this product **>**

- Beads Beating disruption apparatus (for 2 ml tube)
- 70% ethanol
- Chloroform
- Micropipette
- Pipette tip
- 2 ml microtube
- Incubator
- Microcentrifuge
- Vortex mixer

<Standard protocol>

(1) Put 0.2 g of fecal sample in a Beads Tube.
(2) Add 1 ml of Lysis Solution F.
(3) Perform beads beating (4-6 m/sec or 4,200-6,800 rpm for 30-45 sec).(Note 1)
(4) Centrifuge (12,000 x g, 5 min, room temperature).
(5) Transfer 600 µl of the supernatant to a new tube, add 400 µl of Purification Solution, and mix well.(Note 2)
(6) Add 600 µl of chloroform, vortex for 15 sec, and then centrifuge (12,000 x g, 15 min, room temperature).
(7) Transfer 800 µl of the aqueous layer to a new tube while taking care not to transfer the intermediate layer, add 800 µl of Precipitation Solution, mix well, and then centrifuge (20,000 x g, 15 min, 4°C).(Note 3)
(8) Discard the supernatant, add 1 ml of Wash Solution, mix by inverting a few times, and then centrifuge (20,000 x g, 10 min, 4°C).(Note 3), (Note 4)
(9) Discard the supernatant, add 1 ml of 70% ethanol and 2 µl of Ethachinmate, vortex, and then centrifuge (20,000 x g, 5 min, 4°C).(Note 3), (Note 5)
(10) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in 100 µl of TE (pH 8.0).

(Note 1) Make sure that the lid of the Beads Tube is tightly sealed. A loose lid may cause liquid to leak during beads beating.

(Note 2) Since feces absorbs the Lysis Solution F, 600 µl of the centrifuge supernatant may not be recovered. In such cases, scale down the steps (5)-(7) while keeping the ratio of the solutions as it is.
Step (5), (6)
Centrifuge supernatant : Purification Solution : Chloroform = 6 : 4 : 6
Step (7)
Aqueous layer : Precipitation Solution = 1 : 1
No change after Step (8).

(Note 3) If the maximum centrifugal force of the available centrifuge is not more than 20,000 x g, then spin at the maximum centrifugal force (but not less than 12,000 x g).

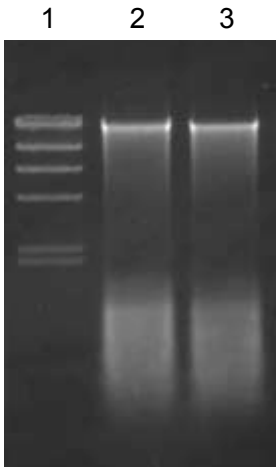
(Note 4) Remove as much of the supernatant as possible.
It is possible that the substances included in the supernatant may inhibit PCR. Also, when the yield of DNA is low, the precipitates are not visible in this step.

(Note 5) Fecal DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol. However, if Ethachinmate is not added, avoid vortexing and gently wash the precipitates, mixing by inversion.

VI Data collection

1. DNA extraction from fecal samples

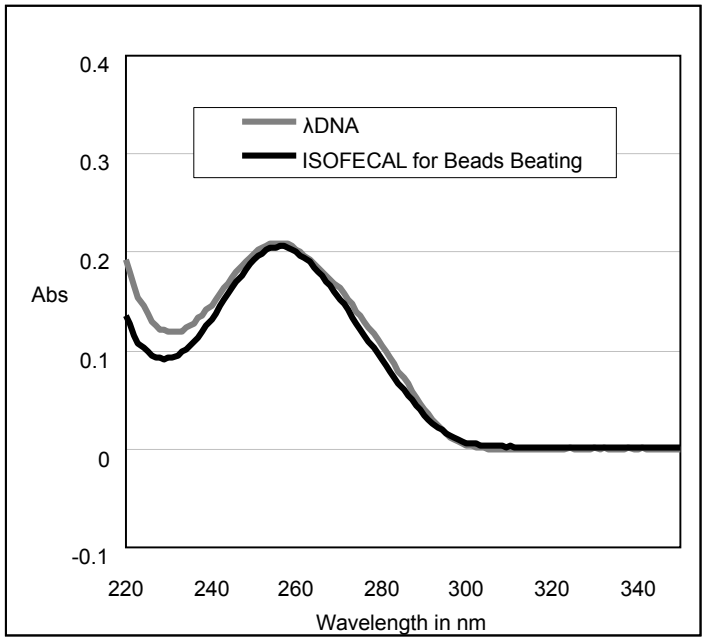
1/200 amount of DNA extracted from 0.2 g of human fecal using this kit was electrophoresed in 1% Agarose S.



Lane 1. OneSTEP Marker 1 (λ/ Hind III digest)
Lane 2. Fecal sample No.1
Lane 3. Fecal sample No.2

2. Absorption spectra of fecal DNA

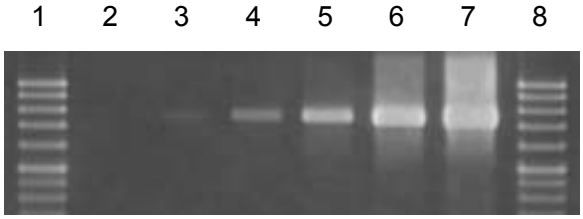
Absorption spectra of DNA extracted from the human fecal using this kit was compared with that of highly purified Lambda DNA (Code No.318-00414).



The fecal DNA extracted with this kit was determined to be high purity.

3. PCR of fecal DNA

Bacterial 16S rRNA gene fragments are amplified using the fecal DNA extracted with this kit as the template, and 1/5 of the amount of the amplified product is electrophoresed in 2% Agarose S.



Lane 1, 8. OneSTEP Ladder 100
Lane 2. No Template Control
Lane 3. Fecal DNA 16 pg
Lane 4. Fecal DNA 80 pg
Lane 5. Fecal DNA 400 pg
Lane 6. Fecal DNA 2,000 pg
Lane 7. Fecal DNA 10,000 pg

VII Troubleshooting

Problem	Possible cause	Possible countermeasure
Low yield of fecal DNA	Few fecal microorganisms	Use fresh fecal whenever possible.
	DNA precipitates may be washed away.	In step (9) of the protocol, the DNA precipitates are prone to be peeled off. Use attached Ethachinmate together with 70% ethanol. Also, since the precipitates become visible by Ethachinmate, carefully remove the supernatant while visually making sure the precipitates are not washed away.
Molecular weight of fecal DNA is low.	DNA is sheared by physical impact of beads beating.	When high molecular weight fecal DNA is desired to be extracted, use ISOFECAL, which employs the heat extraction method in the presence of a surface active agent. However, please note that microorganisms having strong cell walls may not be destroyed using ISOFECAL.
White crystal deposits appear in the Lysis Solution F.	Reagents are precipitated due to low temperature.	Completely dissolve crystals by incubating at 37-65°C and then use. This will not affect quality or performance.
White crystal deposits appear in the Purification Solution.	Reagents are precipitated due to low temperature.	Completely dissolve crystals by incubating at 37-65°C and then use. This will not affect quality or performance.
Floating objects in the Precipitation Solution	Contamination by fungi and the like	Purchase a new kit. Since the composition of this solution allows the growth of fungi and the like, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.

Problem	Possible cause	Possible countermeasure
Floating objects in Wash Solution	Contamination by fungi and the like	Purchase a new kit. Since the composition of this solution allows the growth of fungi and the like, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.
Floating objects in Ethachinmate	Contamination by fungi and the like	Purchase new Ethachinmate. Also, when using, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.
Lysis Solution F spilling out of Beads Tube	The void volume of the fecal is large.	Reduce the amount of fecal sample to less than 0.2 g.

NIPPON GENE CO., LTD.

2-7-18, TOIYA-MACHI, TOYAMA
930-0834 JAPAN
Tel +81-76-451-6548
URL https://www.nippongene.com/

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