Fecal DNA Extraction Kit

ISOFECAL

Manual (First edition)

Code No. 318-06271

NIPPON GENE CO., LTD.

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I Product description

ISOFECAL is a kit for extracting DNA from the fecal samples.
Using this kit, DNA is extracted by the heat extraction method in the presence of a surface-active agent. Thus, DNA extraction from microorganisms having strong cell walls may be difficult. Use this kit for detecting Gram-negative bacteria such as Escherichia coli. If fecal DNA reflecting more actual fecal microbiota needs to be extracted, use ISOFECAL for Beads Beating, which provides chemical lysis by a surface-active agent and physical disruption of cells by beads beating.

II Contents of kit

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 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 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 **>**

- 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 2 ml Tube.
(2) Add 1 ml of Lysis Solution F.
(3) Suspend feces using a toothpick or the like, vortex for 1 min and then incubate at 65°C for 1 hr. (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) Confirm that the feces is completely suspended. Also, we recommend mixing by vortexing or inverting every 10-20 min during the incubation.

(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).

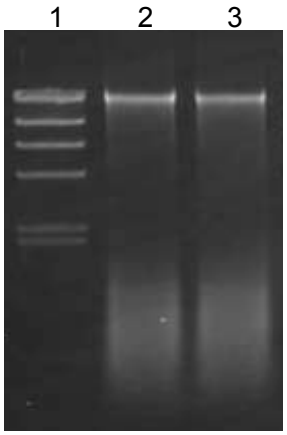
(Note 4) Remove as much of the supernatant as possible.
It is possible that the substances included in the supernatant may inhibit PCR. Also, when the yield of DNA is low, the precipitates are not visible in this step.

(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

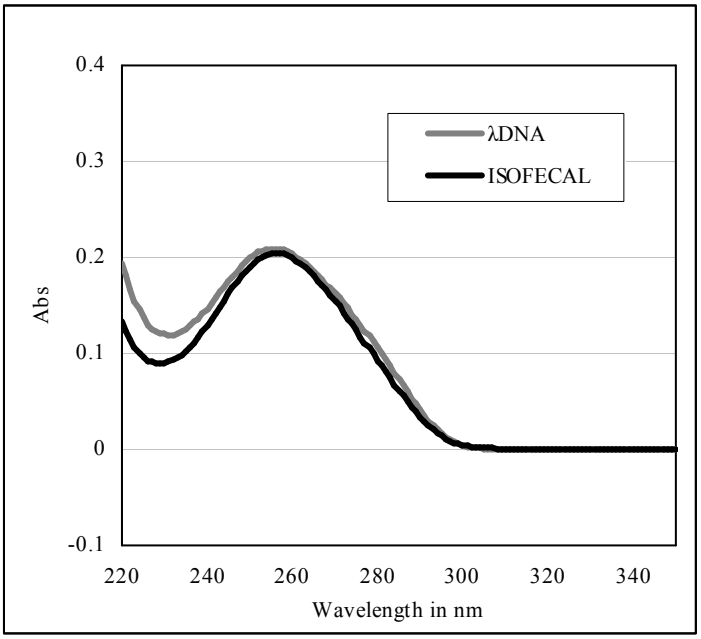
1/200 amount of DNA extracted from 0.2 g of human fecal using this kit was electrophoresed in 1% Agarose S.



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

2. Absorption spectra of fecal DNA

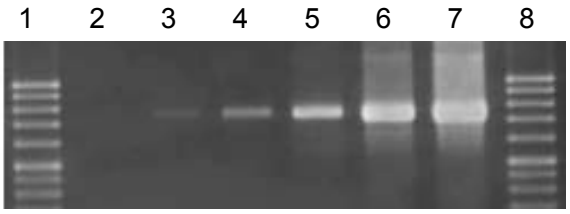
Absorption spectra of DNA extracted from the human fecal using this kit was compared with that of highly purified Lambda DNA (Code No.318-00414).



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.
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.

Problem	Possible cause	Possible countermeasure
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.
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 tube	The void volume of the fecal is large.	Reduce the amount of fecal sample to less than 0.2 g.

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