# FASMAC

For Soil and Fecal DNA

# GenCheck® DNA Extraction kit Type S/F

Product code: GC002-1N

**Instruction Manual** 



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### 1. Product Description

GenCheck® DNA Extraction kit Type S/F is a kit for extracting DNA from soil and fecal samples. With extraction solution of special composition, DNA can be extracted from not only non-volcanic ash soil but also volcanic ash soil that was difficult to extract. GenCheck® DNA Extraction kit Type S/F combines the chemical lysis method with a surfactant and a physical cell disruption method by Beads Beating as the method to extract DNA. As a result, DNA can also be extracted from microorganisms with strong cell walls. Therefore, soil DNA and fecal DNA that reflect the actual microorganism group structure can be obtained. DNA is collected by centrifugation using a membrane based spin column used for collecting DNA. Therefore, DNA can be extracted easily and quickly without preparing nucleic acids by ethanol precipitation. The extracted soil DNA and fecal DNA are suitable for the microbial community structure analysis of the microorganisms present in soil and feces, soil analysis, and the intestinal flora analysis using next-generation sequencing and PCR-DGGE analysis.

### 2. Kit Contents

Size: 50 preps

	Component	Unit	Quantity
1	Lysis Solution M	50 ml	1
2	Lysis Solution S*	2.5 ml	1
3	Purification Solution*	20 ml	1
4	Wash Solution 1	30 ml	2
<b>⑤</b>	Wash Solution 2	30 ml	1
<b>6</b>	TE (pH8.0)	5 ml	1
7	RNase A (100 mg/ml)	100 µl	1
8	Beads Tube	50 pieces	1
9	Spin Column	50 pieces	1
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<sup>\*</sup> Crystals may precipitate in the Lysis Solution S and Purification Solution, but there is no problem with quality and performance. In such cases, dissolve the crystals completely before use by incubating each container at about 65°C (with occasional mixing).

### 3. Storage

Store at room temperature ( $15^{\circ}$ C ~  $25^{\circ}$ C) .However, when using RNase A (100 mg/ml), ensure that there is no contamination (such as fungi and bacteria) and store at a low temperature ( $2^{\circ}$ C to  $10^{\circ}$ C) after opening. When not in use for a long time, cold storage ( $-20^{\circ}$ C) is recommended.

## 4. Requirements in Addition to the Kit

- Bead crusher (for 2 ml tube)
- 100% Ethanol
- Chloroform
- Micropipette
- Pipette tip

- 2 ml micro tube
- Incubator
- Centrifuge
- Vortex mixer
- Timer

### 5. Protocol

- (1) Put 0.5 g soil/stool sample in the **Beads Tube (2.0 ml tube)**.
- (2) Add 950 μl of Lysis Solution M and 50 μl of Lysis Solution S.
- (3) Set Beads Tube of (2) in the bead crusher and crush for 30 to 45 seconds at 4,200 6,800 rpm.
  \*Ensure that the cover of the Beads Tube is firmly closed. Loose cover can cause the solution to leak during Beads Beating.
- (4) Incubate for 1 hour at 65°C.
- (5) Centrifuge (12Kxg, 1 minute, room temperature).
- (6) Transfer 600 μl of the supernatant to a new tube and add 2 ml of **RNase A** (100 mg/ml).
- (7) Incubate for 10 minutes at 37°C.
- (8) Add 400 μl of **Purification Solution** and mix thoroughly.
- (9) Add 600 μl of chloroform, vortex for 15 seconds, and then centrifuge (20Kxg, 15 minutes, room temperature).
  \*If the maximum centrifugal force of the centrifuge used is less than 20Kxg, use the centrifuge with its maximum centrifugal force (however, 12Kxg or more).
- (10) Transfer 800  $\mu$ l of the aqueous layer to a new tube ensuring that the intermediate layer is not transferred. Add 800  $\mu$ l of **100% ethanol** and mix well.
- (11) Add 800 µl including the precipitates to the spin column and centrifuge (13Kxg, 30 seconds, room temperature).
  - \*Discard the filtrate and repeat twice.
- (12) Discard the filtrate, and add 600  $\mu$ l of **Wash Solution 1** to the spin column and centrifuge (13Kxg, 1 minute, room temperature).
  - \*Discard the filtrate and repeat twice.

- (13) Discard the filtrate, and add 600  $\mu$ l of **Wash Solution 2** to the spin column and centrifuge (13Kxg, 1 minute, room temperature).
- (14) Discard the filtrate, and transfer the spin column into a collection container, add **TE (pH8.0)** and incubate for 3 minutes at room temperature.
- (15) Centrifuge (13Kxg, 1 minute, room temperature) and collect the DNA.

## 6. Precautions

- (1) This product is a kit for extracting DNA present in the soil and stool. The product cannot be used for other purposes.
- (2) Only those with a thorough knowledge of the reagents should use this product.
- (3) Use this product as per the instructions given in the Instruction Manual.
- (4) FASMAC cannot assume responsibility in case of problems that may arise because of not following the instructions.
- (5) The expiry date is mentioned on the outer case of the product. Use before the expiry date.
- (6) GenCheck® DNA Extraction kit Type S/F is manufactured and sold under license for the patent owned by Todai TLO Ltd.
- (7) For disposal method, follow the regulations of the country and local government where this product is used.

  \*Material: Tube (PP), bottle (PP), beads (Zirconia), label (PET), outer case (Paper), and manual (Paper)
- (8) Before using, read the information given in SDS. You can download the product SDS from the FASMAC website (http://www.fasmac.co.jp/) .





Sold by & Contact; FASMAC Co., Ltd

5-1-3, Midorigaoka, Atsugi, Kanagawa, 243-0041

TEL; 046-295-8787 FAX; 046-294-3738

Email; gmo@fasmac.co.jp