FavorPrep™ Stool DNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FAST1030 (4 Preps)	FAST1033 (50 Preps)	FAST1034 (100 Preps)
SDE1 Buffer	1.8 ml	20 ml	40 ml
SDE2 Buffer	1 ml	10 ml	20 ml
SDE3 Buffer	1 ml	4 ml	8 ml
SDE4 Buffer (Concentrate)▲	1.5 ml	20 ml	40 ml
Wash Buffer (Concentrate)■	3 ml	17.5 ml	35 ml
Elution Buffer	0.5 ml	5 ml	7 ml
Proteinase K (Liquid)	100 µl	1050 µl	1050 µl x 2
Bead Tubes	4 pcs	50 pcs	100 pcs
HE Columns	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	8 pcs	100 pcs	100 pcs x 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **SDE4 Buffer** and **Wash Buffer** by adding 96~100% ethanol.			
Volume of Ethanol for SDE4 Buffer▲	1.5 ml	20 ml	40 ml
Volume of Ethanol for Wash Buffer■	12 ml	70 ml	140 ml

All kit components are shipped at room temperature and should be stored at room temperature between 15~25°C.

■ Specification

Format/Principle	Spin Column (silica matrix)
Binding Capacity	≤125 µg DNA/Column
Operation Time	<60 mins
Sample Size	≤50 mg (solid stool)/≤200 mg (loose stool)
DNA yield	≤15 µg
Elution Volume	30 µl

Procedure Overview

Stool sample

- Weigh stool sample and add 320 µl SDE1-PK mixture in a Bead Tube.
- Vortex for 5 mins.
- Incubate at 60°C for 15 mins.

Centrifuge
18,000 xg, 1 min

- Mix all the supernatant with 150 µl SDE2 Buffer in a 1.5 ml tube.
- Incubate on ice for 5 mins.

Centrifuge
18,000 xg, 5 mins

- Mix all the supernatant with 50 µl SDE3 Buffer and (Optional) 6 µl RNase A in a 1.5 ml tube.
- Incubate at room temperature for 2 mins.

Centrifuge
18,000 xg, 2 mins

HE Column

- Mix 330 µl supernatant with 660 µl SDE4 Buffer (ethanol contained) in a 1.5 ml tube.
- Transfer all the mixture into HE Column.

Centrifuge
18,000 xg, 1 min

- Add 900 µl Wash Buffer (ethanol contained).

Centrifuge
18,000 xg, 1 min

- Add 500 µl Wash Buffer (ethanol contained) and dry the column membrane.

Centrifuge
18,000 xg, 2 mins

- Add 30 µl Elution Buffer.
- Stand the column for 5 mins.
- Obtain purified genomic DNA.

Centrifuge
18,000 xg, 1 min

■ Preparation Before Starting

1. For faster processing, please follow the **quick protocol** on the official website.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Additional materials: 96~100% ethanol, RNase A (Cat. No. FARA2093; optional).
4. (Optional) For DNA long-term storage, mix stool sample with FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) at a volume ratio of 1:4 (stool : reagent).
5. Set up a water bath or dry bath at 60°C and preheat the Elution Buffer to 60°C for elution step.
6. Check **SDE1 Buffer** before use. If precipitates are observed, warm-up SDE1 Buffer at 60°C until precipitates are completely dissolved.
7. Vortex **SDE3 Buffer** evenly before use.
8. Fresh preparation of **SDE1-PK mixture**, premix 300 µl of **SDE1 Buffer** and 20 µl of **Proteinase K** per sample before executing DNA extraction.
9. (Optional) If RNA-free genomic DNA is required, premix 50 µl **SDE3 Buffer** with 6 µl RNase A (50 mg/ml) per sample before executing DNA extraction.
10. Add indicated volume of ethanol (96~100%) into **SDE4 Buffer** and **Wash Buffer**, mix well and store at room temperature.
11. Recommended amount of sample:

Stool sample	Recommended amount
Loose stool (Higher than 80% water content) e.g., fish, cattle, human diarrhea	50~200 mg (50~200 µl)
Solid stool (50~80% water content) e.g., human, cat, dog	30~50 mg
Dry stool (Low water content) e.g., mouse, reptile	10~30 mg

■ General Protocol

- **Note:** All centrifugation steps should be performed at **18,000 ×g** at room temperature.
- **Note:** Avoid disturbing the pellet or debris while transferring the supernatant.

1. Weigh appropriate stool sample (see the **Recommended amount table**) into a **Bead Tube.** Add 320 µl **SDE1-PK mixture** to the tube.

2. Vortex using horizontal agitation (Horizontal Tube adapter) or a homogenizer to grind the fresh stool for 5 mins. Mix thoroughly and spin down.
   - **Note:** If the sample is frozen, reduce the grinding time from 5 mins to 1 min.
3. Incubate mixture at 60°C for 15 mins until the stool is lysed completely. Vortex occasionally during incubation.
4. Centrifuge for 1 min, then transfer all supernatant carefully into a 1.5 ml tube (not provided).
   - **Note:** If supernatant is less than 200 µl, the amount of sample is exceeded. Please decrease the sample amount or separate sample in multiple preparation.
5. Add 150 µl **SDE2 Buffer** and mix thoroughly by pulse-vortexing. Incubate sample on ice for 5 mins.
6. Centrifuge for 5 mins, then transfer all supernatant carefully into a 1.5 ml tube. (not provided)
7. Add 50 µl **SDE3 Buffer** and **(Optional)** RNase A. Mix thoroughly by pulse-vortexing and incubate sample at room temperature for 2 mins.
8. Centrifuge for 2 mins, then transfer 330 µl supernatant carefully into a 1.5 ml tube. (not provided)
9. Add 660 µl **SDE4 Buffer** (ethanol contained) to the sample mixture, mix thoroughly by pipetting.
10. Place an **HE Column** in an **HE Collection Tube**, then transfer all mixture carefully into the HE Column.
11. Centrifuge for 1 min. Discard flow-through and place the HE Column in a new HE Collection Tube.
12. Add 900 µl **Wash Buffer** (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.
13. Add 500 µl Wash Buffer (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and HE Collection Tube.
14. Place the **HE Column** in an **Elution Tube**, then add 30 µl prewarmed **Elution Buffer** or ddH_2O (pH 7.5~9.0) directly onto the membrane. Stand the HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
15. Centrifuge for 1 min to elute DNA.