FavorPrep™ Stool DNA Isolation Mini Kit

User Manual

For Research Use Only

Cat. No.: FASTI 001 (50 Preps)
FASTI 001-1 (100 Preps)

Introduction

The FavorPrep™ Stool DNA Isolation Kit is designed for isolation of high quality total DNA from 50~200 mg of fresh or forzen stool sample. The inhibitors of the downstream application such as polysaccharides, humic acid, phenolic compounds will be removed by utilizing the DNA binding column and the buffer system in this kit. The entire procedure is not required the phenol-chloroform extraction and can be finished within 60 minutes. The purified DNA is ready for PCR and other downstream application.

Specification:

Sample Size: 50-200 ml of fresh or forzen stool sample

Handing Time: about 60 minutes

Kit Contents

	FASTI001 (50 preps)	FASTI001-1 (100 preps)
Glass Beads	12 g	25 g
SDE1 Buffer	20 ml	40 ml
SDE2 Buffer	7 ml	14 ml
SDE3 Buffer	15 ml	30 ml
SDEX Buffer	20 ml	40 ml
Wash Buffer	20 ml*	35 ml**
Elution Buffer	15 ml	30 ml
Proteinase K +	11 mg	11 mg x 2
SDE Mini Column	50 pcs	100 pcs
2.0 ml Tube (for Collection)	100 pcs	200 pcs
Bead Tube	50 pcs	100 pcs

*Add 80 ml of ethanol (96~100%) to Wash Buffer when first open.

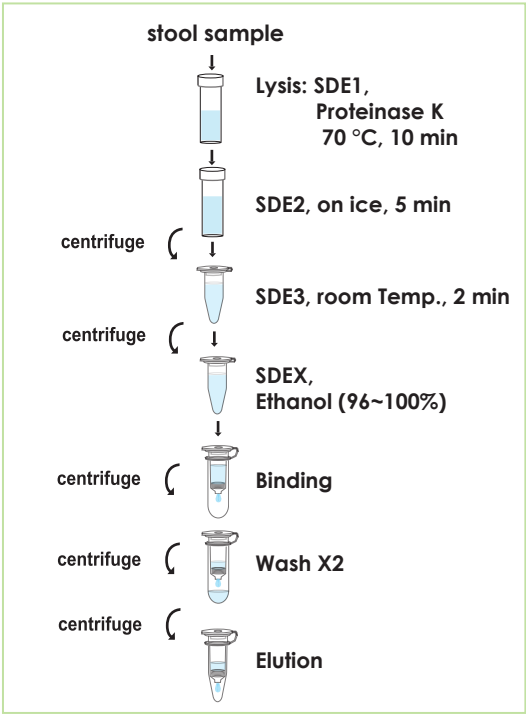
**Add 140 ml of ethanol (96~100%) to Wash Buffer when first open.

+Add 1.1 ml of sterile ddH_2O to Proteinase K tube to make a 10 mg/ml stock solution.

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check SDE1 Buffer before use, Warm SDE1 Buffer at 60°C for 10 minutes if any precipitate formd.
3. Add 1.1 ml of sterile ddH_2O to Proteinase K tube to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. Store the stock solution at 4 °C.
4. Add 80 ml/ 140 ml of ethanol (96-100%) to Wash Buffer when first open.
5. Prepare a water baths to 70 °C before the operation.
6. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.
7. Preheat Elution Buffer or ddH_2O to 60°C for elution step.

Brief Procedure:



General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. **Add 200 mg of Glass Beads into a 2.0 ml Bead Tube (provided). And transfer 50~100 mg of stool sample into Bead Tube then place on ice.**
   --If the sample is liquid, add 200 µl of sample into a 2.0 ml Beads Tube.

2. **Add 300 µl of SDE1 Buffer and 30 µl of proteinase K (10 mg/ml) to the sample, vortex at maximum speed for 5 minutes. Incubate the sample at 70 °C for 10 minutes and vortex the sample twice during the incubation.**
   --Make sure that stool sample is homogenized completely.
   -- For isolation of DNA from gram positive baceria, do a further incubation at 95 °C for 5 minutes.

3. **Briefly spin the tube to remove drops from the inside of the lid.**

4. **Cool down the sample and add 100 µl of SDE2 Buffer to the sample, mix well by vortexing. Incubate the sample on ice for 5 minutes.**

5. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 5 minutes.**

6. **Carefully transfer the supernatant to a 1.5 ml microcentrifuge (not provied) and discard the stool pellet.**
   --Avoid pipetting any debris and pellet.

7. **Add 200 µl of SDE3 Buffer to the sample, mix well by vortexing. Incubate the sample at room temperature for 2 minutes.**
   --Note: SDE3 Buffer must be suspended completely by vigorously vrotexing before every using.
   -- use 1ml pipettor and cut off the end of 1 ml tip to make it easier for pipetting the SDE3 Buffer.

8. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 minutes.**

9. **Carefully transfer 250 µl of supernatant to a clean 1.5 ml microcentrifuge tube (not provied) and discard the pellet.**
   --Avoid pipetting any debris and pellet.

10. **(Optional) If RNA-free DNA is required, add 1 µl of 100 mg/ml RNase A (not provided) to the sample and mix well. Incubate at room temperature for 2 min.**

11. **Briefly spin the tube to remove drops from the inside of the lid.**

12. **Add 250 µl of SDE4 Buffer and 250µl of ethanol (96~100%) to the sample, mix thoroughly by pulse-vortexing.**

13. **Place a SDE Column into a Collection Tube, and transfer all of the sample mixture to the SDE Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through. And place the SED Column to a new Collection Tube.**

14. **Add 750 µl of Wash Buffer (ethanol added) to SDE Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through. And repeat this step for one more time.**
    --Make sure that ethanol (96~100%) has been added into Wash Buffer when first open.

15. **Centrifuge at full speed (14,000 rpm) for an additional 3 min to dry the SDE column.**
    --Important step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

16. **Place the SDE Column into a Elution Tube, Add 50~200 µl of preheated Elution Buffer or ddH_2O to the membrane center of the SDE Column. Stand the SDE Column for 2 min at room temperature.**
    --Important step! For effective elution, make sure that the Elution Buffer or ddH_2O is dispensed onto the membrane center and is absorbed completely.

17. **Centrifuge for 1 min to elute DNA.**

Troubleshooting:

Low yield

- Too many sample were used
  --- reduce the sample volume.
- Poor sample lysis because of insufficient Proteinase K activity
  --- Use a fresh or well-stored Proteinase K stock solution
- Poor sample lysis because of insufficient sample beating with glass beads
  --- Extend the vortexing time at maximum speed
- Poor sample lysis because of insufficient incubation time
  --- Extend the incubation time
- Ethanol is not added into the lysate before transferring into SDE Mini Column
- Ethanol is not added into Wash Buffer when first open; the volume or the percentage of ethanol is not correct before adding into Wash Buffer
- Elution of DNA is not efficient
  --- Make sure the pH of ddH_2O is between 7.5- 8.5.
  --- After Elution Buffer or ddH_2O is added, stand the SDE Mini Column for 2 min before centrifugation.

Column is clogged

- Disrupt the debris pellet when transfer the sample supernatant
- Sample is too viscous
  --- Reduce the sample volume.

Purified DNA dose not perform well in downstream application

- Sample is old
  --- Always use fresh or well-stored sample for stool DNA extraction.
- Residual ethanol contamination
  --- After Wash step, centrifuge at 4,000 x g for an additional 3 minutes to dry the SDE Mini Column.
- RNA contamination