FavorPrep™ Stool DNA Isolation Mini Kit

Cat.No. : FASTI 000, 4 preps
FASTI 001, 50 Preps
FASTI 001-1, 100 Preps
(For Research Use Only)

Kit Contents:

	FASTI 000 (4 preps_sample)	FASTI 001 (50 preps)	FASTI 001-1 (100 preps)
SDE1 Buffer	1.8 ml	20 ml	40 ml
SDE2 Buffer	1.2 ml	7 ml	14 ml
SDE3 Buffer	1.2 ml	15 ml	30 ml
SDE4 Buffer	3 ml	20 ml	40 ml
Wash Buffer (concentrate) *	1.5 ml	20 ml	35 ml
Elution Buffer	1.5 ml	15 ml	30 ml
Proteinase K (lyophilized) *	1.1 mg	11 mg	11 mg x 2
SDE Mini Columns	4 pcs	50 pcs	100 pcs
Collection Tubes	8 pcs	100 pcs	200 pcs
Elution Tubes	4 pcs	50 pcs	100 pcs
Bead tubes	4 pcs	50 pcs	100 pcs
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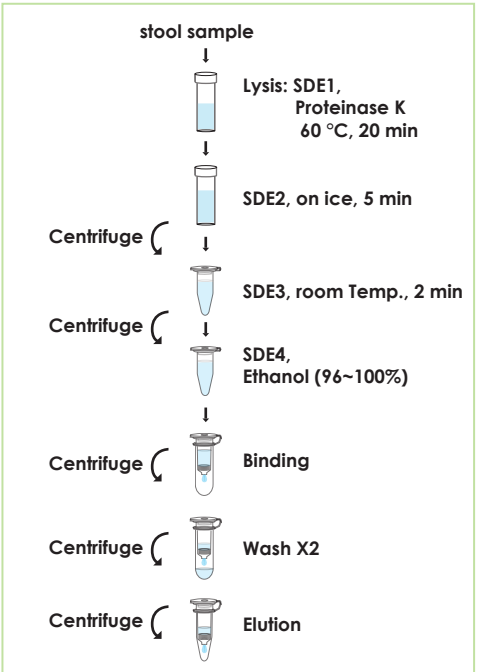
* Preparation of Proteinase K solution and Wash Buffer for first use:

Cat. No:	FASTI 000	FASTI 001	FASTI 001-1
ddH2O volume for Proteinase K Solution	0.11 ml	1.1 ml	1.1 ml
ethanol volume for Wash Buffer	6 ml	80 ml	140 ml

Specification:

Principle: spin column (silica membrane)
Sample: 50 ~ 200 mg
Operation time: < 60 min
Elution volume: 50~200 µl

Brief Procedure:



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 required sterile ddH2O 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 indicated volume of ethanol (96-100%) to Wash Buffer before use.
5. Prepare a heating block or a water bath to 60 °C. If DNA is isolated from gram positive bacteria, prepare a heating block or a water bath to 95 °C for another incubation.
6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
7. Preheat Elution Buffer or ddH2O to 60°C for elution step.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Add up to 200 mg of stool sample to a bead tube and place the tube on ice.
Note: -- If the sample is dry, reduce the sample size to ≤ 50 mg.
-- If the sample is liquid, add 200 µl of sample into a bead tube.

2. Add 300 µl of SDE1 Buffer and 20 µl of proteinase K (10 mg/ml) to the sample. Vortex at maximum speed for 5 minutes. Incubate the sample mixture at 60 °C for 20 minutes and vortex the sample for every 5 minutes during the incubation.
-- Make sure stool sample is homogenized completely.
-- For isolation of DNA from gram positive baceria, do a further incubation at 95 °C for 5 minutes after proteinase K lysis.

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

4. Cool down the sample mixture and add 100 µl of SDE2 Buffer. Mix well by vortexing and incubate the sample mixture on ice for 5 minutes.

5. Centrifuge at full speed (~18,000 x g) for 5 minutes.

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

7. Add 200 µl of SDE3 Buffer. Mix well by vortexing and incubate the sample mixture at room temperature for 2 minutes.
--Note: SDE3 Buffer must be suspended completely by vigorously vrotexing before every using.
-- Cut off the end of 1 ml tip to make it easier for pipetting the SDE3 Buffer.

8. Centrifuge at full speed for 2 minutes.

9. Carefully transfer 250 µl of supernatant to a 1.5 ml microcentrifuge tube (not provied).
--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). Mix well and incubate the sample mixture 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 250 µl of ethanol (96~100%). Mix thoroughly by pulse-vortexing.**
13. **Place a SED Column into a Collection. Transfer all of the sample mixture to the SDE Column. Centrifuge at full speed for 1 min and discard the flow-through then place the SDE Column into a new Collection Tube.**
14. **Add 750 µl of Wash Buffer (ethanol added) to the SDE Column. Centrifuge at full speed for 1 min then discard the flow -through. Return the SDE Column back to the Collection Tube.**
    --Make sure that ethanol (96~100%) has been added into Wash Buffer when first use.
15. **Repeat step 15.**
16. **Centrifuge at full speed for an additional 3 min to dry the SDE column.**
    --Important step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
17. **Place the SDE Column into a 1.5 ml microcentrifuge tube (not provided). Add 50 ~ 200 µl of preheated Elution Buffer or ddH2O 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 ddH2O is dispensed onto the membrane center and is absorbed completely.
18. **Centrifuge at full speed for 1 min to elute DNA.**

Troubleshooting

Problem	Possible reasons	Soutions
Low or no yield of genomic DNA		
	Sample stored incorrectly	Store the stool sample at -20 °C.
	Low amount of cells in the sample	Increase the sample size
	Poor cell lysis	
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.
	Poor cell lysis because of insufficient mixing with SD1, SD2 Buffer and Proteinase K	Mix the sample mixture immediately thoroughly by pulse -vortexing after adding SD1, SD2 Buffer and proteinase K solution.
	Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
	Insufficient binding of DNA to column's membrane	
	Ethanol is not added into sample lysate before DNA binding	Make sure that the correct volumes of ethanol (96- 100 %) is added into the sample lysate before DNA binding.
	Ethanol and sample lysate did not mix well before DNA binding	Make sure that Ethanol and sample lysate have been mixed completely before DNA binding
	Incorrect preparation of Wash Buffer W1/W2	
	Ethanol is not added into Wash Buffer when first use	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer when first use.
	The volume or the percentage of ethanol is not correct for adding into Wash Buffer	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer when first use.
	Elution of DNA is not efficient	
	pH of water (ddH2O) for elution is acidic	Make sure the pH of ddH2O is between 7.0-8.5.
		Use Elution Buffer (provided) for elution .
	Elution Buffer or ddH2O is not completely absorbed by column membrane	After Elution Buffer or ddH2O is added, stand the SD Column for 5 min before centrifugation.
Poor quality of genomic DNA		
A260/A280 ratio of eluted DNA is low	**Poor cell lysis**	
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.
	Poor cell lysis because of insufficient mixing with SD1 buffer, SD2 Buffer and Proteinase K	Mix the sample mixture immediately thoroughly by pulse -vortexing after adding SD1, SD2 Buffer and proteinase K solution.
	Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
A260/A280 ratio of eluted DNA is high	A lot of residual RNA in eluted DNA	Add 8 µl of RNase A (50 mg/ml) to the eluate and incubate at 37 °C for 10 minutes. After incubation, add 200 µl of SD2 Buffer and 200 µl of ethanol (96~100%), mix well by plus -vortexing. Then follow the general Protocol starting from step 7.