



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)	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 (Liquid)	100 µl	1050 µl	1050 µl × 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 Wash Buffer for the first use:

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Ethanol volume for Wash Buffer	6 ml	80 ml	140 ml

Specification:

Principle: spin column (silica membrane)

Sample: 50~200 mg

Operation time: <60 mins

Elution volume: 50~200 µl

Important Notes:

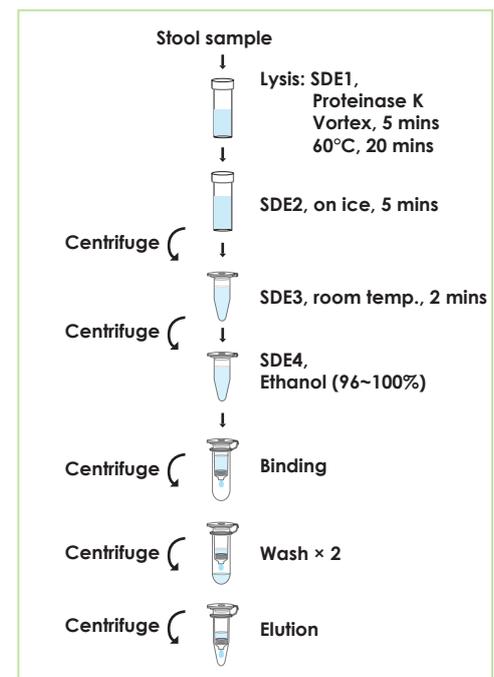
1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check SDE1 Buffer before use, warm SDE1 Buffer at 60°C for 10 mins if any precipitate formed.
4. Add indicated volume of ethanol (96~100%) to Wash Buffer before use.
5. Prepare a heating block 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 to the sample. Vortex at maximum speed for 5 mins. Incubate the sample mixture at 60°C for 20 mins and vortex the sample for every 5 mins during the incubation.
 - Make sure stool sample is homogenized completely.
 - For isolation of DNA from gram positive bacteria, required additional incubation at 95°C for 5 mins after proteinase K lysis.
3. Briefly spin the tube to remove drops which 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 mins.
5. Centrifuge at full speed (~18,000 x g) for 5 mins.
6. Carefully transfer the supernatant to a 1.5 ml microcentrifuge tube (not provided) 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 mins.
 - Note: SDE3 Buffer must be suspended completely by vigorously vortexing 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 mins.
9. Carefully transfer 250 µl of supernatant to a 1.5 ml microcentrifuge tube (not provided).
 - Avoid pipetting any debris and pellet.

Brief Procedure:



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 mins.
11. Briefly spin the tube to remove drops which 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 Tube. 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 at the first use.
15. Repeat step 14.
16. Centrifuge at full speed for an additional 3 mins 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 Elute Tube (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 mins 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	Solutions
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 SDE1, SDE2 Buffer and Proteinase K.	Mix the sample mixture immediately thoroughly by pulse-vortexing after adding SDE1, SDE2 Buffer and Proteinase K.
	Poor cell lysis because of insufficient Proteinase K activity.	Make sure the reactive temperature and time is correct.
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		
	Ethanol is not added into Wash Buffer at the first use.	Make sure that the correct volume of ethanol (96~100%) is added into Wash Buffer at the first use.
	The volume or the percentage of ethanol is not correct for adding into Wash Buffer.	Make sure that the correct volume of ethanol (96~100%) is added into Wash Buffer at the first use.
Elution of DNA is not efficient		
	pH of water (ddH ₂ O) for elution is acidic.	Make sure the pH of ddH ₂ O is between 7.0~8.5. Use Elution Buffer (provided) for elution.
	Elution Buffer or ddH ₂ O is not completely absorbed by column membrane.	After Elution Buffer or ddH ₂ O is added, stand the SDE Column for 5 mins 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 SDE1, SDE2 Buffer and Proteinase K.	Mix the sample mixture immediately thoroughly by pulse-vortexing after adding SDE1, SDE2 Buffer and Proteinase K.
	Poor cell lysis because of insufficient Proteinase K activity.	Make sure the reactive temperature and time is correct.