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FavorPrep™ Soil DNA Isolation Mini Kit

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

Kit Contents:

	FASOI 000 (4 preps_sample)	FASOI 001 (50 preps)	FASOI 001-1 (100 preps)
Glass Beads	1 g	12 g	25 g
SDE1 Buffer	3.6 ml	40 ml	70 ml
SDE2 Buffer	1.2 ml	15 ml	25 ml
SDE3 Buffer	1.2 ml	15 ml	30 ml
SDE4 Buffer	1.5 ml	25 ml	40 ml
Wash Buffer (concentrate) *	1.5 ml	20 ml	40 ml
Elution Buffer	1.5 ml	25ml	50 ml
SDE Mini Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
Bead tube	4 pcs	50 pcs	100 pcs
User Manual	1	1	1

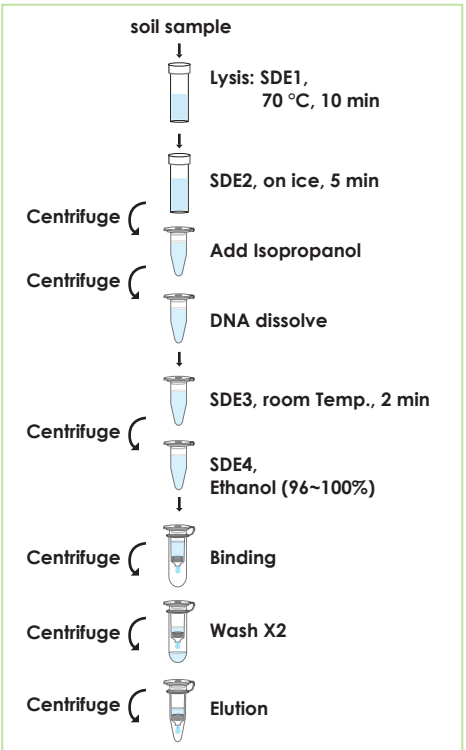
* Preparation of Wash Buffer for first use:

Cat. No:	FASOI 000	FASOI 001	FASOI 001-1
ethanol volume for Wash Buffer	6 ml	80 ml	160 ml

Specification:

Principle: spin column (silica membrane)
Sample: 0.25 ~ 0.5 g
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 indicated volume of ethanol (96-100%) to Wash Buffer before use.
4. Prepare a heating block or a water bath to 70 °C. If DNA is isolated from gram positive bacteria, prepare a heating block or a water bath to 95 °C for another incubation.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
6. Preheat Elution Buffer or ddH2O to 60°C for elution step.

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). Transfer 0.25 ~ 0.5 g of soil 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 600 µl of SDE1 Buffer 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.
--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 mixture and add 200 µl of SDE2 Buffer. Mix well by vortexing. Incubate the sample on ice for 5 minutes.

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

6. Carefully transfer the clarified supernatant to a 1.5 ml microcentrifuge tube (not provied). Measure the volume of the supernatant.
--Avoid pipetting any debris and pellet.

7. Add 1 volume of isopropanol and vortex to mix well. centrifuge at full speed for 10 min to pellet DNA.
-- For example: If the clarified lysate volume is 450 µl, add 450 µl of isopropanol to the clarified lysate.

8. Carefully discard the supernatant and invert the tube on the paper towel for 1 min to remove residual liquid.
--Do not disrupt the pelle.

9. Add 200 µl of pre-heated Elution Buffer or ddH2O, vortex to dissolve the DNA pellet completely.

10. Add 100 µl of SDE3 Buffer to the sample, mix well by vortexing. Incubate the sample at room temperature for 3 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.

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11. **Centrifuge at full speed for 2 minutes.**
12. **Carefully transfer the supernatant to a 1.5 ml microcentrifuge (not provied). And measure the volume of the supernatant.**
    --Avoid pipetting any debris and pellet.
13. **(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.**
14. **Briefly spin the tube to remove drops from the inside of the lid.**
15. **Add 1 volume of SDE4 Buffer and 1 volume of ethanol (96~100%). Mix thoroughly by pulse-vortexing.**
    For example: If the clarified lysate volume is 250 µl, add 250 µl of SDE4 Buffer and 250 µl of ethanol (96~100%) to the sample.
16. **Place a SDE Column into a Collection Tube and transfer all of the sample mixture to the SDE Column. Centrifuge at full speed for 1 min then discard the flow-through then place the SDE Column into a new Collection Tube.**
17. **Add 750 µl of Wash Buffer (ethanol added) to the SDE Column. Centrifuge at full speed for 1 min then discard the flow-through.**
    --Make sure that ethanol (96~100%) has been added into Wash Buffer when first use.
18. **Repeat step 17.**
19. **Centrifuge at full speed for an additional 3 min to dry the SDE column.**
    --Important step! This step will avoid the residual liquid to inhibi subsequent enzymatic reactions.
20. **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.
21. **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.
	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.
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.