

FavorPrepTM
Soil DNA Isolation Midi Kit

User Manual

Cat. No.: FASOI 002 (24 Preps)

For Research Use Only

v.1107

Introduction

The FavorPrep Soil DNA Isolation Midi Kit is designed for isolation of total DNA from 1 ~10 g of soil sample. The inhibitors of the downstream PCR or enzymatic reactions will be removed with the sequent buffers system of this kit. The entire procedure is not required the phenol-chloroform extraction and can be finished within 90 min. The purified DNA is ready for PCR and other downstream application.

Kit Contents

FASOI002 (24 Preps)

Glass Beads	55 g
SDE1 Buffer	200 ml X2
SDE2 Buffer	135 ml
SDE3 Buffer	30 ml
SDE4 Buffer	85 ml
Wash Buffer (concentrated)	40 ml* X2
Elution Buffer	120 ml
SDE Midi Column	24 pcs
Elution Tube (50 ml centrifuge tube)	24 pcs
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*Add 160 ml ethanol (96~100%) to Wash Buffer when first open.

18. Centrifuge at 2,500 ~ 4,000 x g at room temperature for an additional 10 min to dry the SDE Midi column.

--It might be necessary to dry the column further by placing the column in a vacuum oven at 70 °C for 10 minutes.

--**Important step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

19. Place SDE Midi Column into a new 50 centrifuge tube, Add 1~ 2 ml of preheated Elution Buffer or ddH₂O to the membrane center of the SDE Midi Column. Stand the SDE Midi Column for 5 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.

20. Centrifuge at 2,500 x g at room temperature for 2 min to elute DNA.

10. Add 1 ml 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 vortexing before every using.
- Use 1ml pipettor and cut off the end of 1 ml tip to make it easier for pipetting the SDE3 Buffer.

11. Centrifuge at 2,500 x g at room temperature for 5 minutes.

12. Carefully transfer the clarified lysate to a 15 ml or 50 ml microcentrifuge (not provided). And measure the volume of the clarified lysate.

- Avoid pipetting any debris and pellet.

13. (Optional) If RNA-free DNA is required, add 4 µl of 100 mg/ml RNase A (not provided) to the sample and mix well. Incubate at room temperature for 2 min.

14. Add 1 volume of SDE4 Buffer and 1 volume of ethanol (96~100%) to the clarified lysate, mix thoroughly by pulse-vortexing.

For example: If the clarified lysate volume is 2.5 ml, add 2.5 ml of SDE4 Buffer and 2.5 ml of ethanol (96~100%) to the clarified lysate.

15. Place the SDE Midi Column in a 50 ml centrifuge (Ex: Falcon 50 ml) and transfer all of the sample mixture to the SDE Midi Column.

16. Centrifuge at 2,500 x g at room temperature for 2 min then discard the flow-through. Then place the SDE Midi Column back in 50 ml centrifuge tube.

17. Add 7 ml of Wash Buffer (ethanol added) to SDE Midi Column. Centrifuge at 2,500 x g at room temperature for 2 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.

Applications:

PCR , Real-Time PCR
Infection disease research

Sample amount:

Sample: up to 10 g of soil sample

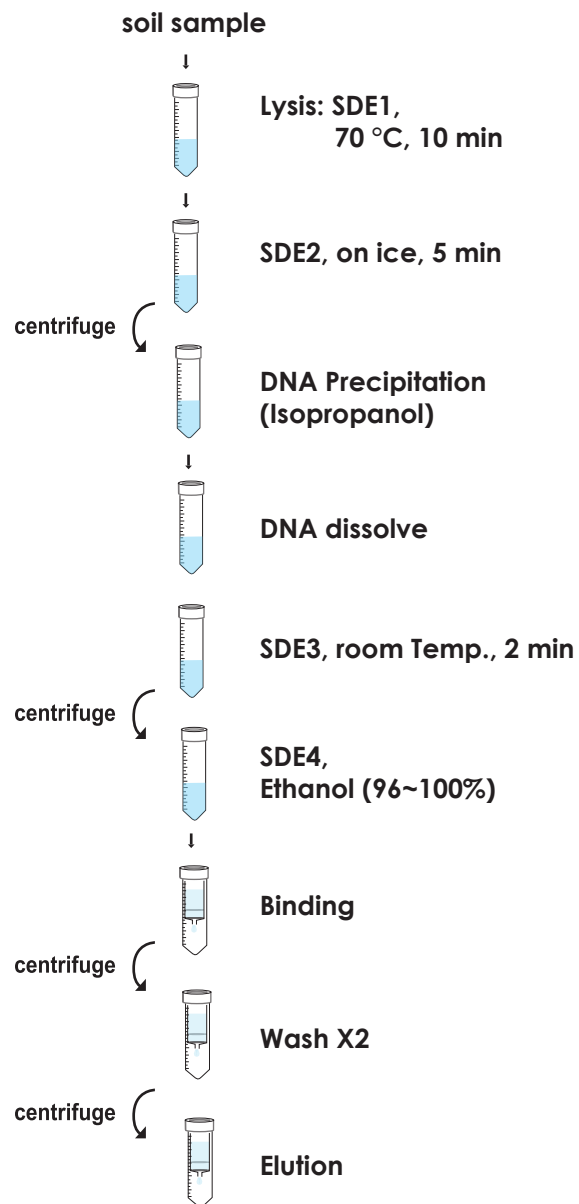
Handling time: 90 minutes

Elution Volume: 2 ml

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 formed.
3. Add 160 ml of ethanol (96-100%) to Wash Buffer when first open.
4. Prepare a water bath to 70 °C before the operation.
5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.
6. Preheat Elution Buffer or ddH₂O to 60°C for elution step.

Brief Procedure



General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer up to 10 g of soil sample to a 50 ml centrifuge tube. (not provided)
2. Weigh 2 g of Glass bead and mix with soil sample.
3. Add 15 ml 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 bacteria, do a further incubation at 95 °C for 5 minutes.
4. Cool down the sample and add 5 ml of SDE2 Buffer to the sample, mix well by vortexing. Incubate the sample on ice for 5 minutes.
5. Centrifuge at 2,500 x g for 5 minutes at room temperature.
6. Carefully transfer the clarified lysate to a 50 ml centrifuge tube (not provided). And measure the volume of the clarified lysate.
--Avoid pipetting any debris and pellet.
7. Add 1 volume of isopropanol, vortex to mix well. centrifuge at 15,000 x g at 4°C for 30 min to precipitate DNA.
-- For example: If the clarified lysate volume is 12 ml, add 12 ml of isopropanol to the clarified lysate.
8. Carefully discard the supernatant and invert the tube on the paper towel for 5 min to remove residual liquid.
--Do not disturb the pellet.
9. Add 2 ml of pre-heated Elution Buffer or ddH₂O, vortex to dissolve the pellet completely.