# FavorPrep™
# Soil DNA Isolation Midi Kit

# User Manual

**Cat. No.: FASOI 002 (24 Preps)**

**For Research Use Only**

v.0905

# 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.**

## Applications:

**PCR , Real-Time PCR**
**Infection disease research**

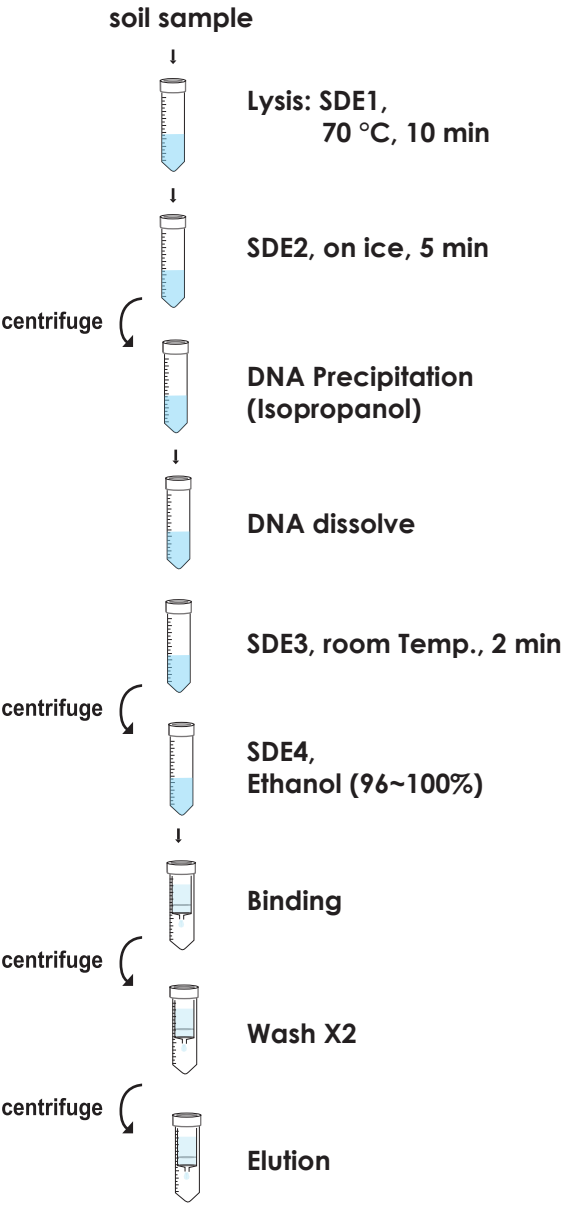
## Sample amount:

**Sample: up to 10 g of soil sample**
**Handing 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 formd.**
3. **Add 6 ml of ethanol (96-100%) to Wash Buffer when first open.**
4. **Prepare a water baths 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 dd$H_2O$ 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 baceria, 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 provied). 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 precipate DNA.**
   **--** For example: If the clarified lysate volume is 12 ml, add 12 ml of isopropanol to the clarified ltsate**.**

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 dd$H_2O$, vortex to dissolve the pellet completely.**

**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 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.

**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 provied). 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 ltsate.

**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.

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 futher 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 dd$H_2O$ 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 dd$H_2O$ 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.**