

## SOIL DNA ISOLATION MINI KIT

## DE-026

Soil DNA Isolation Mini Kit operates through our high-quality beads beating disruption method and is perfect for use with diverse soil samples of up to 0.5 g.

Our silica membrane technology, and spin column along with beads beating method guarantee the high-quality purification and isolation of DNA that can be used for PCR, genotyping, arrays, etc.

Kit Components	DE-025S (4 preps)	DE-025 (50 preps)	DE-026 (100 preps)
SDE1 Buffer	1.8 ml × 2	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	25 ml	50 ml
SDE Mini Column	4 pcs	50 pcs	50 pcs × 2
Collection Tube	8 pcs	100 pcs	100 pcs × 2
Elution Tube	4 pcs	50 pcs	100 pcs
Bead Tube	4 pcs	50 pcs	100 pcs

\* Preparation of Wash Buffer for first use:

Cat. No:	DE-025S	DE-025	DE-026
Ethanol volume for Wash Buffer	6 ml	80 ml	160 ml

### Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves, safety glasses 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 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 xg) in a microcentrifuge.
6. Preheat Elution Buffer or ddH<sub>2</sub>O to 60°C for elution step.

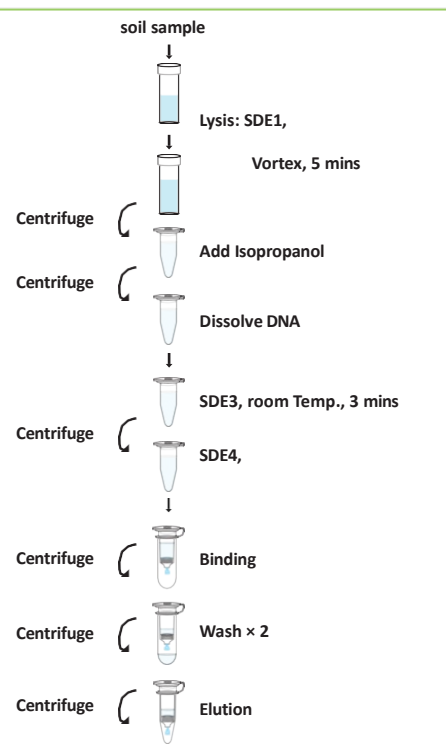
### Specifications:

Principle: spin column (silica membrane)

Sample: 0.25~0.5 g

Operation time: <60 mins

Elution volume: 50~200 µl



### General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer 0.25~0.5 g of soil sample into Bead Tube then place on ice.

--If the sample is liquid, add 200  $\mu$ l of sample into a 2.0 ml Beads Tube.

2. Add 600  $\mu$ l 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, require additional 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  $\mu$ l of SDE2 Buffer. Mix well by vortexing. Incubate the sample on ice for 5 minutes.

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

6. Carefully transfer the clarified supernatant to a 1.5 ml microcentrifuge tube (not provided). 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  $\mu$ l, add 450  $\mu$ l 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 pellet.

9. Add 200  $\mu$ l of pre-heated Elution Buffer or ddH<sub>2</sub>O, vortex to dissolve the DNA pellet completely.

10. Add 100  $\mu$ l 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.

-Cut off the end of 1 ml tip to make it easier for pipetting the SDE3 Buffer.

11. Centrifuge at full speed for 2 minutes.

12. Carefully transfer the supernatant to a 1.5 ml microcentrifuge (not provided) and measure the volume of the supernatant.

-Avoid pipetting any debris and pellet.

13. (Optional) If RNA-free DNA is required, add 1  $\mu$ l 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  $\mu$ l, add 250  $\mu$ l of SDE4 Buffer and 250  $\mu$ l 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  $\mu$ l 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 inhibit subsequent enzymatic reactions.

20.Place the SDE Column into Elute Tube (provided). Add 50~200  $\mu$ l of preheated Elution Buffer or ddH<sub>2</sub>O onto 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 ddH<sub>2</sub>O 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	Solutions
<b>Low or no yield of genomic DNA</b>		
	Sample stored incorrectly	Store the stool sample at -20°C.
	Low amount of cells in the sample	Increase the sample size
<b>Poor cell lysis</b>		
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.
<b>Insufficient binding of DNA to column's membrane</b>		
	Ethanol is not added into sample lysate before DNA binding	Make sure that the correct volumes of ethanol (96~100%) are 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
<b>Incorrect preparation of Wash Buffer</b>		
	Ethanol is not added into Wash Buffer when first use	Make sure that the correct volumes of ethanol (96~100%) are 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%) are added into Wash Buffer when first use.
<b>Elution of DNA is not efficient</b>		
	pH of water (ddH <sub>2</sub> O) for elution is acidic	Make sure the pH of ddH <sub>2</sub> O is between 7.0~8.5. Use Elution Buffer (provided) for elution .
	Elution Buffer or ddH <sub>2</sub> O is not completely absorbed by column membrane	After Elution Buffer or ddH <sub>2</sub> O is added, stand the SDE Column for 5 min before centrifugation.
<b>Poor quality of genomic DNA</b>		
A260/A280 ratio of eluted DNA is low	<b>Poor cell lysis</b>	
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.

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