

ONESpin™ Rapid Soil DNA Extraction Kit (ONE-SLDNA-50)

Principle:

The **ONESpin™ Rapid Soil DNA Extraction Kit** is based on silica spin column–based purification technology for rapid and efficient isolation of high-quality genomic DNA from soil samples.

Soil samples containing microbial cells, plant material, humic substances, and other environmental inhibitors are subjected to mechanical and chemical lysis using a proprietary lysis buffer to release nucleic acids. Insoluble debris and particulate matter are removed during sample clarification by centrifugation.

Under optimized chaotropic salt conditions, genomic DNA selectively binds to the silica membrane of the spin column, while proteins, humic acids, polysaccharides, and other contaminants pass through.

The bound DNA is washed sequentially using wash buffers to remove residual inhibitors and salts. A brief centrifugation step ensures complete removal of wash buffer residues.

Finally, purified DNA is eluted in a low-salt elution buffer and collected in a clean microcentrifuge tube.

The isolated DNA is suitable for downstream molecular applications including PCR, qPCR, microbial community analysis, metagenomics, and next-generation sequencing (NGS).

Kit Contents:

Components	Quantity (50 Reactions)
LM Tubes	50 Tubes
SL Buffer	25 mL
PC Buffer	25 mL
BB Buffer	30 mL
Spin Columns	50
BW Buffer (Concentrate)	15 mL
BE Buffer	3 mL

Material's/Equipment's Required but not Supplied:

- Ethanol (96–100%), Molecular Grade
- Micropipettes and sterile tips
- 1.5 mL Microcentrifuge Tubes
- Heating block or Water bath
- Vortex Mixer
- Microcentrifuge

Storage Conditions:

All components of the **ONESpin™ Rapid Soil DNA Extraction Kit** should be stored at room temperature (~25°C). The kit is stable until the expiration date printed on the product label when stored under recommended conditions.

Preparation of Buffers

Preparation of BW Buffer

BW Buffer is supplied as a **concentrate** and must be diluted with ethanol before use.

Component	Volume for 50 Reactions
BW Buffer (Concentrate)	15 mL
Ethanol (96-100%)	45 mL
Final Volume	60 mL

Mix thoroughly and store at room temperature.

Protocol:

1. Weigh up to 250 mg of soil sample into an LM tube containing ceramic beads.
2. Add 400 μ L of SL Buffer and 400 μ L of PC Buffer, then vortex vigorously for 5 minutes to ensure efficient mechanical lysis.
3. Incubate at 65 °C for 15 minutes.
4. Invert the tube several times during incubation to mix the sample.
5. Centrifuge at 8,000 rpm for 8 minutes and transfer 500 μ L of lysate to a new 1.5 mL tube.
6. Add 500 μ L of BB buffer and vortex well.
7. Transfer the mixture to a spin column placed in a collection tube.
8. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
9. If the sample volume exceeds the column capacity, repeat step 7–8 until all the lysate has been processed.
10. Add 500 μ L of BW buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
11. Repeat the wash step once more with BW buffer.
12. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
13. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
14. Add 50 μ L of BE buffer directly to the center of the membrane.
15. Incubate at room temperature for 5 minutes.
16. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

Troubleshooting Guide:

Problem Observed	Possible Cause	Recommended Solution
Low DNA yield	Incomplete lysis of soil sample	Ensure thorough vortexing (5 min). Increase lysis time to 20–25 min for difficult samples.
	Insufficient mixing with SL & PC buffer	Vortex immediately after adding buffers; ensure uniform suspension.
	Loss of lysate during transfer	Carefully pipette supernatant without disturbing pellet.
	Elution inefficiency	Pre-warm BE buffer (60°C) and increase incubation time to 10 minutes before elution.
No DNA or very low concentration	Ethanol not added to BW buffer	Confirm BW buffer was prepared with ethanol correctly.
	Column clogging	Reduce input soil amount (≤ 200 mg for clay-rich soil).
	Incorrect centrifugation speed	Ensure recommended rpm (convert to $\sim 8,000$ – $10,000 \times g$ if needed).
Column clogging / slow flow-through	High debris or particulate matter	Increase centrifugation time in step 5 or perform additional clarification spin.
	Overloading of sample	Reduce soil input amount.
DNA degradation	Nuclease contamination	Use sterile, nuclease-free consumables. Work quickly.
	Improper storage	Store eluted DNA at -20°C immediately.
Presence of inhibitors (PCR inhibition)	Carryover of humic acids	Perform additional BW wash step.
	Incomplete washing	Ensure both wash steps are performed properly.
	Residual ethanol in column	Perform proper dry spin (Step 12). Increase time if needed.
Poor PCR/qPCR performance	Inhibitor contamination	Dilute DNA sample (1:10) before PCR.
Eluted DNA volume too low	Incomplete elution	Ensure BE buffer is added to membrane center.
	Membrane not fully wetted	Increase incubation time before centrifugation.
Inconsistent results between samples	Uneven lysis or mixing	Standardize vortex time and mixing steps.
	Variation in soil type	Adjust input amount and lysis conditions based on soil type (clay, sandy, organic).