Note: *for laboratory research use only*

Soil DNA Rapid Extraction Kit (Spin-column)

Kit for the isolation of genomic DNA from soil/silt/feces

Cat. # **DP4001 (50 preps)**

Signalway Biotechnology

I. Kit Content, Storage and Stability

Content	Storage	50 preps (DP4001)
Buffer A	-20℃	750 μl
Buffer B	RT	5 ml
Buffer C	RT	25 ml
Protein Precipitation Buffer	RT	18 ml
Elution Buffer EB	RT	5 ml
Purification Column	RT	50
Collection Tube	RT	50

All reagents are stable for 12 months if stored properly.

Reminder:

1. Buffer A contains protease K, and please store it at -20℃.
2. Buffer B may precipitate under low temperature. Incubate at 65°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
3. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

II. Principle

In this kit, innovative extraction and lysis system are utilized for rapidly lysing cells and inactivating cellular nuclease in soil. The treatment of glass beads is not used and the integrity of genomic DNA is guaranteed. DNA purity is greatly ensured from efficiently removing debris and contaminants by specialized DNA spin-column. Innovative washing solution removes trace contaminants, and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without any further purification.

III. Features

1. Rapid DNA isolation (about 1 hour).
2. Suitable for various soils and stools.
3. Isolated high-quality DNA is suitable for all kinds of molecular biology experiments, such as PCR, Southern-blot, and restriction enzyme digests.
4. Excellent yield and recovery (up to 50 μg from 0.3-0.5 g sample).

IV.Notes

Read this section before your experiment

1. All the centrifugation steps can be performed at RT.
2. Buffer B contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes and cloth. **If contact occurs, wash with water or physiological saline.**
3. **The final yield of genomic DNA depends on type, size, bacteria or organism species and the concentration.**
4. Agarose gel electrophoresis or a UV－spectrometer can be used for detecting the concentration and purity of the DNA. The result may display multiple bands at different sizes on your agarose gel. These multiple bands are influenced by organisms and extracting methods.
5. Digest to check your DNA size when compared with a DNA marker.
6. The value of OD_{260}/OD_{280} of DNA achieves 1.7-1.9.
7. There is no EDTA in Buffer EB, which should not affect down-stream reactions. If interfere occurs, use sterile water for DNA; however, please ensure pH >7.5, and then store DNA at -20℃. For long-term storage, dissolve DNA in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and make sure to dilute the DNA solution before use because the EDTA will affect down-stream reactions.
8. **Isopropanol** or **70% ethanol** should be prepared by user.

V. Procedure

Check the Buffer B for precipitate; incubate at 65℃ until clear if necessary!

Please keep Buffer lid tightly caped after use!

1. Load 0.3-0.5 g fresh sample to a centrifuge tube. Add 1 ml Buffer EB and 5 µl Buffer A, and then vortex for 1-2 minutes. Mix thoroughly and then put into 37℃ water bath for 10 minutes.

   (Mix thoroughly every 2-3 minutes)

2. Add 100 µl Buffer B and mix thoroughly for 1-2 minutes. Put into 65℃ water bath for 10 minutes.

   (Mix thoroughly every 2-3 minutes)

3. Centrifuge at 10,000 rpm for 10 minutes and harvest supernatant to a new 1.5 ml centrifuge tube.
4. Add 300 µl Protein Precipitation Buffer and mix thoroughly. .
5. Put into ice bath for 8 minutes, and then centrifuge at 13,000 rpm for 10 minutes and harvest supernatant.
6. Add 500 µl Buffer C in the middle of Purification Column, hold for 1 minute, and then centrifuge at 10,000 rpm for 1 minute.
7. Add the supernatant from step 5 into spin-column P, centrifuge at 1,600 rpm for 1 minute in traditional centrifuge, e.g. *eppendorf 5417R*. **Harvest the flow through, which contains DNA**.
   - **Please follow the centrifuge speed instruction; otherwise, the DNA yield will be affected.**
8. Add 800 µl isopropanol to the flow-through from step 7. Mix thoroughly and then centrifuge at 13, 000 rpm for 10 minutes.
9. Carefully remove upper suspension and invert the tube for 2 minutes for air dry.
10. Use 30 µl Buffer EB dissolve the precipitation. Transfer all to a new centrifuge tube.

    (If the precipitation is not clean enough, use 800 µl 70% ethanol to wash twice*, and then use the Buffer EB to dissolve it.

*Use 70% ethanol to wash the pellet and then centrifuge at 13, 000 rpm for 5 minutes, and then remove the upper suspension.)

11. Store DNA in -20℃ or apply to down-stream reactions.