Note: *for laboratory research use only*

Stool Genomic DNA Isolation kit

(Spin-column)

kit for the isolation of genomic DNA from soil/silt/stool

Cat. # **DP4601 (50 preps)**
DP4602 (100 preps)
DP4603 (200 preps)

Signalway Biotechnology

I. Kit Content, Storage and Stability

Content	Storage	50preps (DP4601)	100preps (DP4602)	200 preps (DP4603)
Buffer ET	RT	50 ml	100 ml	200 ml
Buffer A	-20℃	750 µl	1.5 ml	1.5ml x2
Buffer B	RT	5 ml	10 ml	20 ml
Buffer PE	RT	18 ml	36 ml	72 ml
Buffer C	RT	25 ml	50 ml	100 ml
Buffer EB	RT	5 ml	10 ml	20 ml
Spin-column P (with collection tube)	RT	50 pcs	100 pcs	200 pcs

All reagents are stable for 12 months when stored properly.

Reminder:

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

II. Principle

In this kit, innovation extraction and lysis system are utilized for rapidly lysing cells and inactivating cellular nuclease. 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. (Around 40 minutes)
2. Compatible, suitable for various soils and stools.
3. Isolated high-quality DNA is suitable or directly used for all kinds of molecular biology experiments like PCR, Southern-blot and restriction enzyme digests.
4. High yield and recovery (up to 50μg/rection, 0.3-0.5g sample).

IV.Notes

1. **All the centrifugation steps can be performed at RT** in a traditional centrifuge.
2. Buffer B contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes and cloth**. If contact occurs then wash with water or physiological saline.**
3. PCR inhibitor will be removed during procedure.
4. **The final yield of genomic DNA depends on type, size, bacteria or organism species and the concentration.**
5. 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 organism and extracting methods.
6. Digest to check your DNA size when compared with a DNA marker.
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 at -20℃. For long-term storage, dissolve DNA in TE (10mM Tris-HCl, 1mM 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 solution for precipitate; incubate at 65℃ until clear if necessary!

Please keep Buffer lid tightly caped after use!

1. Load accurate weight 0.3-0.5g fresh sample to a new centrifuge tube. Add 1ml Buffer ET 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 10,000rpm for 5 minutes and harvest supernatant to a new 1.5 centrifuge tube.
4. Add 300 µl Buffer PE and mix thoroughly. .
5. Put into ice bath for 8 minutes, and then centrifuge at 13,000rpm for 5 minutes and harvest supernatant. .
6. Add 500 µl Buffer C in the middle of spin-column P, hold for 1 minute, and then centrifuge at 10,000rpm for 1 minute.
7. Add the supernatant from step 5 into spin-column P, centrifuge at 2,000g or 1,600rpm for 1 min in traditional centrifuge, e.g. *eppendorf 5417 R*. **Harvest the flow through, which contains DNA**.
   - **Please follow the centrifuge speed instruction, or 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, 000rpm for 5 minutes.
9. Carefully remove upper suspension and invert the tube for 2 minutes and 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, 000rpm for 5 minutes, and then remove the upper suspension.

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