For DNA purification from stool samples

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EXTRACTION Stool DNA Kit

Cat. no. SC08

Kit Contents

Contents	SC0801 50 preps
Buffer GSL	80 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
Proteinase K	1 ml
InhibitEX Tablets	50
Spin Columns	50
Collection Tubes 2 ml	50
Handbook	1

Storage

EXTRACTION Stool DNA Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.

Introduction

EXTRACTION Stool DNA Kit is based on silica membrane technology and special buffer system with InhibitEX Tablet for stool sample gDNA extraction. The spin colu

mn made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes impurity, proteins and other organic compound. Special InhibitEX Tablet can absorb impurity from sample easily. The gDNA isolated with these products is of high quality, high purity and full-length.

Purified gDNA is ready for use in downstream applications such as PCR.

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size and amount.
2. If precipitates formed in Buffer GSL or Buffer GB, warm buffer to 37°C until precipitates fully dissolve.
3. All centrifugation steps should be carried out in a conventional table-top micro centrifuge at room temperature.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Weigh 180-220 mg stool in a 2 ml microcentrifuge tube (not provided) and place the tube on ice.
   Note: If the sample is liquid, pipet 200 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier.
2. Add 1.4 ml Buffer GSL to each stool sample. Vortex continuously for 1 min until the stool sample is thoroughly homogenized.
3. Heat the suspension for 5 min at 70°C.
   Note: The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

4. Vortex for 15 s, centrifuge at 12,000 rpm (~13,400 × g) for 1 min, transfer 1.2 ml supernatant into a new 2 ml centrifuge tube.
5. Add one InhibitEX Tablet to each sample and vortex until the tablet is completely suspended. Incubate the suspension for 1 min at room temperature to allow inhibitors to be adsorbed to the InhibitEX matrix.
6. Centrifuge at 12,000 rpm (~13,400 × g) for 3 min.
7. Transfer supernatant from step 6 into a new 1.5 ml **microcentrifuge tube**. Repeat step 6.
8. Transfer 200 µl supernatant into a new 1.5 ml microcentrifuge tube. Add 15 µl proteinase K.
9. Add 200 µl Buffer GB, and vortex for 15 s.
10. Incubate at 70°C for 10 min to yield a homogeneous solution.

    Note: Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
11. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex.

    Note: Briefly centrifuge the 1.5 ml micro centrifuge tube to remove drops from the inside of the lid.
12. Pipet the mixture into the Spin Column CR2 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the Spin Column CR2 into the collection tube.
13. Add 500 µl Buffer GD to Spin Column CR2 (**Ensure that ethanol is added to Buffer GD before use**), and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
14. Add 600 µl Buffer PW to Spin Column CR2 (Ensure that ethanol is added to Buffer PW before use), and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through

and place the spin column into the collection tube.

15. Repeat Step 14.
16. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.

    Note: The resident ethanol of Buffer PW may have some influence in downstream application.
17. Place the Spin Column CR2 in a new clean 1.5 ml microcentrifuge tube, and pipet 50 µl Buffer directly to the center of the membrane. Incubate at room temperature for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g). Collect the elution.

    Note: For enhancing recovery efficiency of gDNA, pipet the flow through from step 17 into CR2 again, centrifuge for 2 min at 12,000 rpm (~13,400 × g). The pH value of eluted buffer will influence the eluting efficiency; we suggest use Buffer TB or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage, eluting gDNA in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.