TIANamp Stool DNA Kit

For DNA purification from stool samples

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TIANamp Stool DNA Kit
(Spin Column)

Cat. no. 4992205

Kit Contents

Contents	4992205 50 preps
Buffer SA	30 ml
Buffer SC	5 ml
Buffer SH	10 ml
Buffer GFA	10 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
Proteinase K	1 ml
RNase A (10 mg/ml)	600 µl
1 mm Grinding Beads	15 g
RNase-Free Spin Columns CR2	50
Collection Tubes 2 ml	50
Handbook	1

Storage

TIANamp Stool DNA Kit should be kept in dry place and can be stored 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

TIANamp Stool DNA Kit is based on silica membrane technology for stool sample gDNA extraction. The spin column is made of new type silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes impurity, proteins and other organic compound. 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 SA or Buffer SC, warm buffer to 37°C until precipitates fully dissolve.
3. We suggested that the sample should be mixed throughly. If the sample is not mixed well, it may affect the lysis efficiency and ultimately affect the yield and ratio.
4. If the sample absorbs much water, the amount of Buffer SA and Buffer SC can be increased in equal proportion. If Buffer SA and SC are insufficient, they can be purchased separately.
5. 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%), and Buffer GFA Buffer has been prepared with appropriate volume of isopropanol, 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 phase, pipet 200 µl into the microcentrifuge tube.
2. Add 500 µl Buffer SA, 100 µl Buffer SC, 15 µl Proteinase K, 0.25 g Grinding Beads together into the sample. Vortex continuously for at least 10 min until the stool sample is thoroughly homogenized. Using TGrinder H24 Tissue Homogenizer will reduce the lysis time to 1 min and can lyse 24 samples at the same time. Please inquire us for detail information.
3. Heat the suspension for 15 min at 70°C, during which vortex continuously for 2-3 times.
   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 sec, and then centrifuge at 12,000 rpm (~13,400 × g) for 3 min. Transfer the supernatant into a new 2 ml centrifuge tube. Add 10 µl RNase A, mix throughly and incubate at room temperature for 5 min.
5. Add 200 µl Buffer SH, mix throughly and incubate on ice for 5 min.
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. Add equal volume of Buffer GFA **(Ensure that isopropanol is added to Buffer GFA before use)**.
8. Pipet the mixture into the RNase-Free Spin Column CR2 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard flow-through and place the Spin Column CR2 into the collection tube.
9. Add 500 µl Buffer GD to Spin Column CR2 **(Ensure that ethanol (96-100%) is added to Buffer GD before use)**, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and place the spin column into the collection tube.
10. Add 700 µl Buffer PW to Spin Column CR2 **(Ensure that ethanol (96-100%) is added to Buffer PW before use)**, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and place the spin column into the collection tube.
11. Repeat Step 10.

12. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min, discard the flow-through and incubate the Spin Column CR2 at room temperature for several minutes to dry the membrane completely.
    Note: The resident ethanol of Buffer PW may have some influence in downstream application.
13. Place the Spin Column CR2 in a new clean 1.5 ml microcentrifuge tube, and pipet 50 µl Buffer TB 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: To enhance the recovery efficiency of gDNA, pipet the flow through from step 13 into CR2 again, and 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.