EasyPure® Stool Genomic DNA Kit

Cat. No. EE301

Storage: at room temperature (15-25°C) in dry for one year

Description

EasyPure® Stool Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from 180-220 mg of fresh or frozen stool. Solid or liquid stool sample that is rich in contaminants and inhibitors is lysised by the unique lysis buffer. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, qPCR and Next Generation Sequencing.

- Simple and fast.
- Complete removal of contaminants and inhibitors.
- Column based purification, no organic extraction or ethanol precipitation.

Starting material

Fresh or frozen solid or liquid stool, avoiding repeated freezing and thawing.

Kit Contents

Component	EE301-01 (50 rxns)
Lysis Buffer 21 (LB21)	60 ml
Precipitation Buffer 21 (PB21)	15 ml
Binding Buffer 21 (BB21)	35 ml
Clean Buffer 21 (CB21)	6 ml
Wash Buffer 21 (WB21)	12 ml
Elution Buffer (EB)	10 ml
Glass Beads	12.5 g
Genomic Spin Columns with Collection Tubes	50 each
Nuclease-free Tube (2 ml)	100 each
Collection Tubes	150 each

Procedures

Before starting, add the indicated volume of 100% ethanol into the concentrated CB21 and WB21.

Component	EE301-01 (50 rxns)
Clean Buffer 21 (CB21)	24 ml
Wash Buffer 21 (WB21)	48 ml

All centrifugation steps are carried out at room temperature.

1. Add the 180-220 mg of stool, 0.25 g glass Beads and 1 ml of LB21 into a centrifuge tube. Mix well by vortexing, and then incubate in 70°C for 10 minutes.
   Optional: If RNA-free genomic DNA is required, add 20 µl of RNase A for 3-minute incubation at room temperature before 70°C incubation.
2. Centrifuge the tube at 15,000×g for 2 minutes, transfer the supernatant to a new 1.5 ml or 2 ml centrifuge tube.
3. Add 250 µl of PB21 and mix by vortexing. Then incubate on ice for 5 minutes.

4. Centrifuge at 15,000×g for 2 minutes. Transfer the supernatant not exceeding 600 µl to a new 2 ml centrifuge tube (A small amount of precipitation does not affect the next step of the experiment).
5. Add the ethanol and BB21 of the same volume as the supernatant obtained in step 5 (for example: supernatant 600 µl, add 600 µl BB21 and 600 µl ethanol). Mix by vortexing (There may be white flocculent precipitates here).
6. Add 650 µl solution to a spin column, and centrifuge at 12,000×g for 30 seconds. Repeat until all the solution is added to the spin column.
7. Add 500 µl CB21 (check to make sure ethanol has been added). Centrifuge at 12,000×g for 30 seconds. Discard the flow through.
8. Add 500 µl WB21 (check to make sure ethanol has been added). Centrifuge at 12,000×g for 30 seconds. Discard the flow through.
9. Repeat step 8 once.
10. Centrifuge at 15,000×g for 2 minute to remove any residual WB21.
11. Place the spin column to a new sterile 1.5 ml centrifuge tube. Add 50-200 µl of the Elution Buffer or deionized water (pH >7.0) to the center of column. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the genomic DNA.

Notes

- The stool sample stored in ethanol is recommended to be centrifuged to remove ethanol and washed with sterilized water for 2-3 times before use.
- Use sterile tubes and pipette tips to avoid contamination from DNase.
- To obtain more DNA, it is recommended to preheat EB or deionized water at 65°C before elution.

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