Data sheet

Stool DNA Isolation Kit

Cat. No: AN0130 (50 reactions)
Cat. No: AN0131 (100 reactions)

Description

Stool DNA Isolation Kit provides a simple and convenient technique to isolate high quality DNA from fresh or frozen stool samples. Extraction is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts as guanidinium thiocyanate. Fecal samples are rapidly and efficiently lysed by bead beating. The sample DNA is then bound to the surface of a Spin Filter membrane and washed and the bound DNA is then desorbed from the surface of the Spin Filter column. The inhibitors of the downstream PCR will be removed by utilizing the DNA binding column and the buffers system in this kit.

Applications

All molecular biology applications, such as:

- Digestion with restriction enzymes.
- Automated sequencing.
- PCR template.
- Southern Blots.

Quality Certifications

Stool DNA Isolation Kit is tested for isolation of DNA from stool sample. The quantity and quality of purified DNA attend to:

- Ratio 260/ 280.
- Agarose gel electrophoresis.
- Digestion with restriction endonucleases

Kit Components

	AN0130	AN0131
Minispin columns	50	100
Collection tubes (2 mL)	100	200
Dry bead tube	50	100
1.5 mL microcentrifuge tube	50	100
Glass Beads	12 g	25 g
Lysis Solution 1 (LS1)	20 ml	40 ml
Buffer A	15 ml	25 ml
Inhibitor Removal Buffer (IR-Buffer)	15 ml	30 ml
Buffer B	20 ml	40 ml
WB1 Buffer*	20 ml	40 ml
EB buffer	20 ml	40 ml
Proteinase K(20 mg/ml)	1 ml	2 ml

*Add the volume ethanol (96%-100%) specified [Not included] to WB1 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

Kit Storage:

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K at -20ºC, all other kit components can be stored at room temperature. The kit components are stable for 1 year, if stored properly.

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

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Assay procedure

1. Add 200 mg of Glass Beads into a 2.0 ml Bead Tube (provided). And transfer 50-100mg (or 200 µl for liquid sample) of stool sample into Bead Tube and place on ice.
2. Add 300µl of Lysis Solution 1 (LS1) and 20 µl of proteinase K (20 mg/ml). Vortex for 5 minute at maximum speed. *Make sure that stool sample is homogenized completely.*
3. Incubate the sample at 70 °C for 5 minutes. *Votex the sample twice during the incubation.*

   For detection of human DNA it is sufficient to incubate at 70ºC. If necessary, the temperature can be increased to 95°C for isolation of DNA from bacteria or parasites.
4. Spin the tube to remove drops from the inside of the lid.
5. Cool down the sample and add 100 µl of **Buffer A** to the sample, mix well by vortexing. Incubate the sample on ice for 5 minutes.
6. Centrifuge at full speed for 5 minutes. Pipet the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. *Avoid pipetting any debris and pellet.*
7. Add 200 µl of **IR-Buffer** to the sample, mix well by vortexing. Incubate the sample at room temperature for 2 minutes. *IR-Buffer must be suspended completely by vigorously vortexing before every using.*
8. Centrifuge at full speed for 2 minutes. Carefully pipet 250µl of supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. *Avoid pipetting any debris and pellet.*
9. Spin the tube to remove drops from the inside of the lid.
10. Add 250µl of **Buffer B** and 250 µl of ethanol (96%~100%). Mix thoroughly by vortexing.
11. Assemble a spin column with one of the provided collection tubes. Apply all of the sample mixture onto the spin column. Close the cap and centrifuge at full speed for 1 min. Discard the flow-through and reassemble the spin column to a new Collection Tube.
12. Carefully open the spin column and add 750 µl Buffer WB1 (ethanol added). Close the cap and centrifuge at full speed for 1 min. Place the spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate. Repeat this step for one more time.
13. Centrifuge at full speed for an additional 3 min to dry the spin column.

    This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
14. Place the spin column into a new 1.5 mL microcentrifuge tube. Carefully open the spin column and Add 50~200 µl of Elution Buffer or ddH_2O to the membrane center. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.
15. The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.