

Hieff™ Stool DNA Kit

Product description

The Hieff™ Stool DNA Kit is suitable for genomic DNA extraction from various types of stool samples. The silica-based material used in the centrifuge adsorption column is a proprietary new material developed by our company, which, in conjunction with our unique buffer formulation, effectively removes various inhibitory factors present in animal feces that can affect downstream experiments, and efficiently recovers genomic DNA from stool. The extracted DNA has high purity and stable quality, making it suitable for various downstream applications such as restriction digestion, PCR, library construction, and more.

Specifications

Cat.No.	18820ES08 / 18820ES50 / 18820ES70
Size	5 T / 50 T / 200 T

Components

Category	Components No.	Name	18820ES08	18820ES50	18820ES70
Part I	18820-A	Proteinase K (20 mg/mL)	0.1 mL	1 mL	1 mL×4
Part II	18820-B	Hieff DNA Column S2	5	50	200
	18820-C	2 mL Collection Tube	5	50	200
	18820-D	AC Buffer	0.5 mL	5 mL	20 mL
	18820-E	Lysis Buffer /LB Buffer S2	7 mL	70 mL	280 mL
	18820-F	DS agent S2	0.5 mL	5 mL	20 mL
	18820-G	Protein Removal Buffer S2*	1.6 mL	16 mL	64 mL
	18820-H	Binding Buffer /BD Buffer S2	1.1 mL	11 mL	40 mL
	18820-I	Wash Buffer*	1.3 mL	13 mL	50 mL
	18820-J	Elution Buffer	1 mL	10 mL	20 mL

Storage

Part I components should be stored at 2~8°C for 2 years.

Part II components should be stored at room temperature, valid for 2 years.

Notes

1. To prevent evaporation, oxidation, and pH changes caused by prolonged exposure to air, tightly close the cap of each solution immediately after use.
2. Lysis Buffer LB and Binding Buffer BD may precipitate at low temperatures. If precipitation occurs, incubate at 70 °C until the solution becomes clear before use. This will not affect performance.
3. The Impurity Removal Reagent and Binding Buffer BD contain irritants. For your safety, wear a lab coat and

disposable gloves when handling these reagents.

4. This product is for research use only!

Instructions

Preparation Before Experiment

1. Required Materials and Equipment: Benchtop centrifuge, vortex mixer, water bath or metal bath, 1.5 mL centrifuge tubes, absolute ethanol, etc.
2. All centrifugation steps are performed at room temperature using a standard benchtop centrifuge capable of reaching 12,000 rpm.
3. Prior to first use, add absolute ethanol to **Protein Removal Buffer PL* (18820-G)** at a ratio of 1.6:1 (e.g., add 1 mL of absolute ethanol to 1.6 mL of Protein Removal Buffer PL; add 10 mL of absolute ethanol to 16 mL of Protein Removal Buffer PL; add 40 mL of absolute ethanol to 64 mL of Protein Removal Buffer PL). Additionally, add 4 volumes of absolute ethanol to the **Wash Buffer W* (18820-I)** bottle, mix thoroughly before use, and label the bottle accordingly. If the volume of Wash Buffer W* is significantly inaccurate due to improper shipping or storage, measure and adjust the volume using a graduated cylinder before adding 4 volumes of absolute ethanol. After each use, tightly close the cap to maintain the ethanol content in Wash Buffer W*.

Operating Procedure

I. Sample Preprocessing:

1. Take approximately 200 mg of stool, transfer it to a 2 mL centrifuge tube, and place it on ice.
2. Add 1.4 mL **Lysis Buffer S2(18820-E)** vortex for 1 min, and mix thoroughly.
[Note] : If the stool sample is frozen, do not thaw it before adding **Lysis buffer S2**.
[Note] : To ensure thorough mixing, the vortexing time can be appropriately extended.
3. 70°C water bath or metal bath for 5 min.
[Note] : For stool samples containing Gram-positive bacteria that are difficult to lyse, the temperature can be increased to 95°C.
4. Vortex mix for 15 sec, then let sit at room temperature for 1 min. Centrifuge at 12,000 rpm for 1 min to pellet the stool particles. Transfer 900 µL of the supernatant to a 1.5 mL centrifuge tube.
5. Add 100 µL **DS agent S2(18820-F)** and immediately vortex mix until thoroughly homogenized. Let sit at room temperature for 1 min, then centrifuge at 12,000 rpm for 3 min to remove impurities.
6. Transfer the supernatant to a 1.5 mL centrifuge tube and centrifuge at 12,000 rpm for 3 min.
7. Transfer 210 µL of supernatant from step 6 into a 1.5 mL centrifuge tube, add 20 µL **Proteinase K (20 mg/mL)**, and mix thoroughly.
8. Add 200 µL **Binding Buffer S2(18820-H)**, immediately vortex to mix, and incubate in a water bath or metal bath at 70°C for 10 min.

[Note] : To achieve a higher yield, more supernatant can be transferred, while proportionally increasing the amounts of **Proteinase K** (20 mg/mL), **Binding buffer S2**, and subsequent isopropanol.

II. DNA Extraction:

1. Place the DNA Spin Column S2 into a 2 mL collection tube and set aside.
2. Add 100 µL **AC Buffer(18820-D)** to the DNA Binding Column S2, centrifuge at 12,000 rpm for 1 min, and discard the waste liquid.
3. Add 100 µL of isopropanol (user-supplied) to the above pre-cooled sample preparation mixture, **immediately**

vortex to mix, and briefly centrifuge to collect the liquid from the lid of the collection tube.

【Note】 : Flocculent precipitate may form, which is a normal phenomenon.

4. Transfer the mixture to the DNA Spin Column S2, centrifuge at 12,000 rpm for 1 min, and discard the flow-through.
5. Add 500 μ L of **Protein Removal Buffer S2*(18820-G)**, ensure absolute ethanol has been added), centrifuge at 12,000 rpm for 30 sec, and discard the flow-through).
6. Place **Hieff DNA Column S2** back into the collection tube, add 600 μ L of **Wash buffer*(18820-I, ensure absolute ethanol has been added)**, centrifuge at 12,000 rpm for 30 sec, and discard the waste liquid.
7. Repeat step 6.
8. Place **Hieff DNA Column S2**, back into the collection tube, centrifuge the empty column at 12,000 rpm for 2 min at room temperature to remove residual **Wash buffer***.
9. Place **Hieff DNA Column S2** into a new 1.5 mL centrifuge tube, add 50-150 μ L **Elution Buffer(18820-J)** to the center of the membrane, and let it sit at room temperature for 5 min. Then centrifuge at 12,000 rpm for 1 min. Collect the filtrate, which is the DNA solution.

【Note】 : The recovery yield can be improved by the following method: ① Preheat Elution Buffer to 65°C; ② Reapply the DNA filtrate to the column, and let it stand at room temperature for 2 minutes before eluting.

10. The DNA solution can be stored at -20°C for long-term preservation.