High-Q™-Spin-Column Stool DNA Purification Kit

Ordering info

TBK0288, 3 reactions (sample) TBK0289, 50 reactions TBK0290, 200 reactions

Description

High-Q™-Spin-Column Stool DNA Purification Kit provides a convenient method to isolate total DNA from fresh or frozen stool samples. The kit can also be used to isolate DNA from stool samples preserved using our Stool Collection and Preservation Kit.

The stool sample is homogenized and disrupted under denaturing conditions using a specially formulated lysis buffer. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated and removed using a proprietary inhibitor removal buffer. Genomic DNA in the sample is then bound by the Spin Column followed two rapid wash steps to remove trace contaminants, and pure DNA is eluted with Elution Buffer.

Features

- Fast, easy and cost-effective protocol.
- Eliminates PCR inhibitors including humic acids.
- High quality DNA ($A_{260/280}$= 1.8 ± 0.2; $A_{260/230}$= 1.8 ± 0.2)
- Safe, no phenol extraction.
- Expected Yield: 3-15 µg, based on the quality and quantity of the starting material utilized.

Applications

DNA obtained is suitable for downstream molecular biology applications such as PCR techniques, microbiome analysis (NGS), sequencing, restriction enzyme digestion, hybridization methods.

Quality Control

DNA isolation is checked by integrity (agarose electrophoresis), quantity and quality.

Kit Components

Components	TBK0289	TBK0290
High-Q™ Spin Columns with Collection Tubes	50	200
BStool-1 Buffer	55 mL	210 mL
BStool-2 Buffer	15 mL	55 mL
BStool-3 Buffer	16 mL[a]	60 mL[b]
WB2 Buffer	12 mL[c]	45 mL[d]
Elution Buffer	10 mL	25 mL
Proteinase K[e]	30 mg	4 x 30 mg
Proteinase K Resuspension Buffer	1.5 mL	4 x 1.5 mL

Order Info Kit Components: *High-Q™ Spin Column with Collection Tubes (TBM0010) | BStool-1 Buffer (TBB0557) | BStool-2 Buffer (TBB0558) | BStool-3 Buffer (TBB0559) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510) | Proteinase K (TBZ0303) | Proteinase K Resuspension Buffer (TBB0546).*

¡Components for samples are ready to use!

Before its use:

[a] Add 24 mL isopropanol and mix by shaking.

[b] Add 90 mL isopropanol and mix by shaking.

[c] Add 48 mL absolute ethanol and mix by shaking.

[d] Add 180 mL absolute ethanol and mix by shaking.

[e] Dissolve the **30 mg Proteinase K** in **1.5 mL of Proteinase K Resuspension Buffer** and store at –20°C. It is recommended to do several aliquots to avoid many thaw/freeze cycles.

Storage

Store the kit at 25°C. Store Proteinase K at -20°C.

Technical Assistance

Please refer any technical questions to info@tiarisbiosciences.com

PROTOCOL

A. SAMPLE

- **Stool Sample:** Weight **180 -200 mg of stool sample** in a 2 mL microcentrifuge tube (not provided). Add **1 mL BStool-1 Buffer** and vortex briefly (~1-2 minutes) to mix stool and Lysis Solution. *You must completely mix the sample for maximum lysis.*
- **Sample Included in Preservation Solution:** Transfer **1 mL Preserved Sample** into a 2 mL microcentrifuge tube (not provided). *Cut the end of the pipet tip to make pipetting easier.*

B. DNA PURIFICATION

1. Homogenize by continuously shaking at 1,000 rpm for 10 minutes.
2. Incubate at 70 °C for 10 minutes.

   For detection of human DNA, it is sufficient to incubate at 70°C. If necessary, the temperature can be increased to 95°C to isolate DNA from bacteria or parasites.
3. Keep at room temperature for 1 minute.
4. Centrifuge at 13,000 x g for 2 minutes.
5. Carefully aspirate **600 µL supernatant** to a new 1.5 mL microcentrifuge tube (not provided).

   A surface layer of debris may be present on top. Do not to disturb the pellet or transfer any debris.
6. Add **100 µL BStool-2 Buffer** and mix by vortex. Incubate at 4°C for 5 minutes.
7. Centrifuge at 13,000 x g for 5 minutes and take **600 µL of clear supernatant.**

   A layer of fat may appear on the surface. Avoid to take it!
8. Add **25 µL Proteinase K (20 mg/mL)** and incubate at 70 °C for 10 minutes.
9. If a precipitated is observed, centrifuge at 13,000 x g for 1 minute. Transfer the supernatant to a clean 1.5 mL microcentrifuge tube.
10. Add **600 µL BStool-3 Buffer** and mix by inversion or pipetting.

    Check isopropanol has been added to BStool-3 Buffer.
11. Transfer up **700 µL mixture** to a High-Q™ Spin Column placed into a Collection Tube.
12. Centrifuge at 10,000 x g for 1 minute. Remove the flow-through and place back the High-Q™ Spin Column into the Collection Tube. Repeat steps 11 and 12 with the remaining mixture.
13. Add **500 µL WB2 Buffer**. Centrifuge at 10,000 x g for 1 minute. Remove the flow-through and place back the High-Q™ Spin Column into the Collection Tube.

    Check absolute ethanol has been added to WB2 Buffer.
14. Repeat step 13 once.

    Note: *The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If you need to use new collection tubes for each individual step, contact us for information on how to place order.*
15. To dry Spin Column and eliminate residual ethanol, centrifuge again at 10,000 x g for 1 minute.

16. Place the High-Q™ Spin Column into a clean 1.5 mL Tube (not provided) and add **50-100 µL prewarmed Elution Buffer** directly to the center of the membrane.
17. Incubate at room temperature for 2 minutes. Centrifuge at 10,000 x g for 1 minute to elute purified DNA.
18. The purified genomic DNA can be stored at -20°C.