# EasyPrep Stool Genomic DNA Kit

**For DNA purification from stool samples**

# Contents

# Introduction

The EasyPrep Stool Genomic DNA Kit is used for stool sample gDNA extraction. The system is based on silica membrane technology and a special buffer system. The spin column is made of a new type of silica membrane and can bind DNA optimally under given salt and pH conditions. Straightforward centrifugation completely removes impurities, proteins, humic acid, and other organic compounds. The high-quality, high-purity, and full-length gDNA purified by this kit is ready for use in downstream applications such as PCR.

## Important Notes

1. Repeated freezing and thawing of stored samples should be avoided because this leads to a reduction in DNA size and amount.
2. If precipitates form in the Buffer GSA or Buffer GSC, warm the buffer to 37°C until the precipitates fully dissolve.
3. To ensure high lysis efficiency, mix the stool sample and buffer provided by this kit thoroughly.
4. Increase Buffer GSA and Buffer GSC proportionally when dealing with watery stool samples (Buffer GSA and Buffer GSC can be purchased separately).

# Kit Content

| Contents | DPT-BC28 (50 preps) |
|---|---|
| Buffer GSA | 30 ml |
| Buffer GSC | 5 ml |
| Buffer GSH | 10 ml |
| Buffer SFA | 10 ml |
| Buffer SD | 13ml |
| Wash Buffer | 15 ml |
| Elution Buffer | 15 ml |
| Proteinase K | 1 ml |
| RNase A (10 mg/ml) | 600 µl |
| 2 mm Grinding Beads | 40 g |
| Spin Columns GR2 | 50 |
| Collection Tubes（2 ml） | 50 |

Storage

The EasyPrep Stool Genomic DNA Kit can be stored dry at room temperature (15°C–25°C) for up to 12 months with no detriment to performance and quality. For longer storage, the kit should be stored at 2°C–8°C.

# Protocol

**Ensure that Buffer SD and Wash Buffer have been prepared with the appropriate volume of ethanol (96%–100%) and shake thoroughly.**

**Buffer SFA should be added to an appropriate volume of isopropanol (see label).**

***Add 17 mL of ethanol into Buffer SD and add 60 mL of ethanol into the Wash Buffer.***

1. Put a 180–220 mg stool in a 2 mL microcentrifuge tube (not provided) and place the tube on ice.

Note: If the sample is liquid, pipette 200 µL into the microcentrifuge tube. Cut the end of the pipette tip to make pipetting easier.

2. Add 500 µL of Buffer GSA, 100 µL of Buffer GSC, 15 µL of proteinase K, and 0.5 g of beads to each stool sample. Vortex for 30 s and stop for 30 s. Repeat this procedure again until the stool sample is thoroughly homogenized.
3. Heat the suspension for 15 min at 70°C (vortex 2 to 3 times during incubation).

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 s, centrifuge at 12,000 rpm (≈13,400 × *g*) for 3 min, and transfer 1.2 mL of supernatant into a new 2-mL collection tube. Add 10 µL of RNase A to the tube and vortex thoroughly. Incubate the tube at room temperature for 5 min.
5. Add 200 µL of Buffer GSH to each sample and vortex thoroughly. Place the tubes on ice for 5 min.
6. Centrifuge at 12,000 rpm (≈13,400 × g) for 3 min.
7. Transfer the supernatant from step 6 into a new 1.5-mL microcentrifuge tube. Add an equal volume of Buffer SFA into the tube.
8. Pipette the mixture from step 7 into Spin Column GR2 (in a 2-mL collection tube) and centrifuge at 12,000 rpm (≈13,400 × g) for 30 s. Discard the flow-through and place the spin column back into the collection tube.
9. Add 500 µL of Buffer SD to Spin Column GR2 (ensure that ethanol is added to Buffer SD before use), centrifuge at 12,000 rpm (≈13,400 × g) for 30 s, discard the flow-through, and finally place the spin column back into the collection tube.
10. Add 700 µL of Wash Buffer to Spin Column GR2 (ensure that ethanol is added to the Wash Buffer before use), centrifuge at 12,000 rpm (≈13,400 × g) for 30 s, discard the flow-through, and finally

place the spin column back into the collection tube.

11. Repeat step 10.
12. Centrifuge at 12,000 rpm (≈13,400 × g) for 2 min to dry the membrane completely. Discard the flow-through and dry the GR2 column at room temperature for 3–5 min.

Note: The residual ethanol of the Wash Buffer may affect downstream applications.

13. Place the Spin Column GR2 into a new clean 1.5-mL micro centrifuge tube, and pipette 50 µL of Elution Buffer 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).

Note: To enhance the recovery efficiency of gDNA, pipette the flow-through from step 13 into GR2 again. The pH value of the elution buffer will affect DNA recovery; if distilled water is used to elute gDNA, the pH should be 7.0–8.5. Elution efficiency is reduced if the pH is below 7.0. For long-term DNA storage, store the gDNA at −20°C.