



## Genorise Stool DNA Purification Mini Kit 500

This kit is to isolate total DNA including bacterial DNA from solid or liquid stool for 500 applications. If a smaller or larger sample volume is employed, the reagent quantity should be proportionally decreased or increased. This kit can significantly increase quality and quantity of DNA and is much cheaper than the similar products.

Materials provided in the kit:

Genorise Stool DNA Mini Kit	500
Cat No.	GRC101082-500
DNA binding columns	500
Collection Tubes	500
Buffer PBS	160mL
Buffer GS L	155mL
Buffer G L	110mL
Buffer GW1	80mL +50mL ethanol ×2
Buffer GW2	38mL +92mL ethanol ×5
Buffer GE	105mL
Proteinase K(20mg/mL)	1.25mL×7

Materials required but not provided in the kit:

Ethanol

### Protocol

1. Weigh up to 200 g solid stool in a 2 mL tube. If the stool is liquid, directly absorb 200  $\mu$ L to the tube.
2. Add 300 $\mu$ L PBS to the tube, and mix for 5 min on a shaker until no large particles exist.  
▲If you can not fully spread, it may be appropriate to increase the volume of Buffer PBS
3. Add 300 $\mu$ L Buffer GSL to the tube, and mix well, Incubate for 10 min at 70°C.  
▲If you detect DNA in Gram-positive bacteria .Incubate for 5 min at 90°C
4. Centrifuge at 12000 rpm for 1 min. Transfer 200 $\mu$ L supernatant to a new tube.
5. Add 20  $\mu$ L Proteinase K to the tube.
6. Add 200 $\mu$ L Buffer GL to the tube, and mix well, Incubate for 10 min at 70°C.
7. Add 200 $\mu$ L of ethanol (95–100%), Gently flip 4-6 times and mixed well, brief centrifugation.

8. Add the mixture to the DNA column, centrifuge at  $8000 \times g$  for 30 seconds
9. After centrifugation, Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid. Combine the DNA binding column with the Collection Tube.
10. Add 500  $\mu\text{L}$  Buffer GW1. Removal Buffer to the upper reservoir of the Filter Tube. Centrifuge 1 min at  $8,000 \times g$ .
11. Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid. Combine the DNA binding column with the Collection Tube.
12. Add 600  $\mu\text{L}$  Buffer GW2 to the upper reservoir of the DNA binding column. Centrifuge 1 min at  $8,000 \times g$  and discard the flowthrough.
13. Add 600  $\mu\text{L}$  Buffer GW2 to the upper reservoir of the DNA binding column. Centrifuge 1 min at  $8,000 \times g$  and discard the flowthrough.
14. After discarding the flowthrough liquid: Centrifuge the DNA binding column for additional 1 min at full speed. Discard the Collection Tube.  
▲ The extra centrifugation time ensures removal of residual Wash Buffer.
15. Insert the DNA binding column into a clean, sterile 1.5 mL microcentrifuge tube.
16. Add 50  $\mu\text{L}$  prewarmed Buffer GE to the upper reservoir of the DNA binding column. Centrifuge the tube assembly for 1 min at  $10,000 \times g$  1min.
17. Either use the eluted DNA directly or store the eluted DNA at  $-20^{\circ}\text{C}$  for later analysis.

**Note**

1. Warm up the Buffer GE to  $70^{\circ}\text{C}$  to increase the final DNA concentration in the eluate.
2. If you wish to remove RNA from the eluted DNA treat your sample as follows:  
Add to the RNase A 2  $\mu\text{L}$  (10mg/mL) and incubate as appropriate ( $37^{\circ}\text{C}$ , 15min).