For research use only Version Number：3.0-2203

**Stool DNA Isolation Kit**

For genomic DNA purification from cultured cells and animal tissues

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| Kit content | DE-05711 | DE-05712 | DE-05713 | DE-05714 | DE-05715 |
| 5T | 25T | 50T | 100T | 250T |
| Buffer SL1 | 3.5ml | 18ml | 35ml | 70ml | 175ml |
| Buffer SL2 | 3.5ml | 18ml | 35ml | 70ml | 175ml |
| Buffer SL3 | 3.5ml | 18ml | 35ml | 70ml | 175ml |
| Buffer PW | 3ml | 15ml | 30ml | 60ml | 150ml |
| Buffer WB | 2.5ml | 12.5ml | 25ml | 50ml | 125ml |
| Buffer EB | 1.5ml | 6ml | 15ml | 25ml | 75ml |
| Buffer TE | 6ml | 30ml | 60ml | 120ml | 300ml |
| Foregene Protease | 280μl | 1.4ml | 1.4mlx2 | 5.6ml | 14ml |
| Lysozyme | 50mg | 250mg | 500mg | 500mgx2 | 500mgx5 |
| DNA-Only Column | 5 | 25 | 50 | 100 | 250 |
| Instruction manual | 1 | 1 | 1 | 1 | 1 |

**Introduction**

This kit provides a quick and easy method to extract genomic DNA of bacteria and food residues from stool samples of various sources. There are a large number of inhibitors in stool samples. Even if these substances are present in a small amount in the purified DNA, they will affect downstream reactions, such as PCR and restriction enzyme digestion. Therefore, the key to stool DNA purification is to effectively remove inhibitors in stool.

This kit uses DNA-Only Column that can specifically bind DNA, a brand-new Foregene Protease and a unique buffer system, which can effectively remove various inhibitors in stool, without organic solvent extraction or ethanol and isopropanol precipitation. The DNA extraction operation from stool samples can be completed in 40 minutes.

**Storage and Stability**

1. This kit can be stored for 12 months under dry conditions at room temperature (15-25°C); if it needs to be stored for a longer period of time, it can be stored at 2-8°C.

Note: If stored at low temperature, the solution is prone to precipitation. Before use, be sure to place the solution in the kit at room temperature for a period of time. If necessary, preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate, and mix well before use.

1. Foregene Protease solution has a unique formula, which is active when stored at room temperature for a long time (3 months), its activity and stability will be better when stored at 4°C, so it is recommended to store it at 4°C, remember not to keep it at -20°C.
2. Dry Lysozyme powder should be stored at -20°C, the prepared Lysozyme solution should be divided into small portions and stored at -20°C.

**Note: （Be sure to read the notes carefully before using）**

* The appropriate amount of genomic DNA extraction for each stool sample is 50-200mg.
* Buffer SL2 should be thoroughly mixed before use to form a suspension, otherwise it will affect the quality and yield of DNA.
* Before use, carefully check whether there is any precipitation in Buffer SL3 and Buffer PW. If there is precipitation, please dissolve them at 37°C and mix well before use.
* Before using the kit, be sure to check whether Buffer WB is added with anhydrous ethanol according to the instructions. Before use, add 6ml anhydrous ethanol (DE-05711), 30ml anhydrous ethanol(DE-05712), 60ml anhydrous ethanol (DE-05713), 120ml anhydrous ethanol (DE-05714), 300ml anhydrous ethanol (DE-05715) into Buffer WB.
* During the lysis of the sample, keep the sample immersed in the lysis buffer at all time. If the sample adheres to the cap and inner wall of the tube, it can be processed by short-term centrifugation.
* Elution volume: Buffer EB should not be less than 60μl, otherwise it will affect the DNA yield.
* Remember not to add RNase to any buffer.
* All centrifugation steps are centrifuged at room temperature (15-25℃) in a benchtop centrifuge.
* All experimental steps are carried out at room temperature (15-25℃).

**Procedure**

Please add anhydrous ethanol to Buffer WB before use. Please refer to the label on the bottle for the added volume.

1. Weigh **50-200 mg** of stool samples into a 2 ml centrifuge tube.

Note: If the sample is a suspension, centrifuge at 13,300rpm (17,000×g) for 1min, collect the precipitate and weigh 50-200mg into a 2ml centrifuge tube.

1. Add **1ml** Buffer TE and **100μl** Lysozyme (refer to page 9 for preparation method) into the centrifuge tube, mix well, and incubate at 37℃ for 10min (rotation speed: 180rpm).
2. After the warm bath, centrifuge at 13,300rpm (~17,000 ×g) for 1 min, and remove the supernatant with a pipette.

Note: The residual supernatant should be aspirated as much as possible to avoid affecting subsequent operations.

1. Add **600μl** Buffer SL1 and **50μl** Foregene Protease to the centrifuge tube with the pellet left, and mix thoroughly by inversion.
2. Place the centrifuge tube in a 65°C water bath or metal bath for 5 minutes, invert it upside down and mix thoroughly once.

Note: The centrifuge tube needs to be shaken vigorously for about 5 sec for inversion and mixing, until the sample in the centrifuge tube is free of clumping, so that the sample and the lysate can fully react. Otherwise, the yield and purity of DNA will be affected.

1. Centrifuge at 13,300rpm (~17,000 ×g) for 1 min. Transfer the supernatant to a new 2ml centrifuge tube with a pipette. Avoid sucking the pellet and discard the centrifuge tube with the pellet.
2. Add **600μl** Buffer SL2 to the centrifuge tube with the supernatant, invert it upside down and mix thoroughly, and let it stand at room temperature for 2 minutes.

Note: Buffer SL2 needs to be thoroughly mixed before use to form a suspension without layering or clumping. Otherwise, the yield and purity of DNA will be affected.

1. Centrifuge at 13,300rpm (~17,000 ×g) for 1min, transfer **600μl** supernatant to a new 2ml centrifuge tube with a pipette, avoid sucking the pellet.

Note: The remaining supernatant can be discarded or transferred to a new centrifuge tube by adding Buffer SL3 and ethanol by volume for steps 9, 10 and 11. For example, if the remaining supernatant is 400μl, add 400 Buffer SL3 in proportion to carry out step 9, and then add 160μl ethanol to carry out step 10, and then pass through the column.

1. Add **600μl** Buffer SL3 to the centrifuge tube, invert up and down to mix well, place in a 65°C water bath or metal bath for 5 minutes, invert up and down and mix thoroughly once.
2. Add **240μl** ethanol (96-100%) to the centrifuge tube, vortex to mix well for 10 sec, and centrifuge briefly to collect the droplets attached to the tube cap and tube wall.
3. Put the spin column（DNA-Only Column) into the collection tube, add **800μl** of the mixture to the spin column (DNA-Only Column), centrifuge at 12,000rpm (~13,400×g) for 1 min, and discard the waste liquid in the collection tube.
4. Put the spin column back into the collection tube, add the remaining mixture to the spin column, centrifuge at 12,000rpm (~13,400 ×g) for 1 min, and discard the waste liquid in the collection tube.
5. Put the spin column back into the collection tube, add **500μl** Buffer PW to the spin column, centrifuge at 12,000rpm (~13,400 ×g) for 1 min, and discard the waste liquid in the collection tube.
6. Put the spin column back into the collection tube, add **700μl** of Buffer WB to the spin column, centrifuge at 12,000rpm (~13,400 ×g) for 1 min, and discard the waste liquid in the collection tube.
7. Repeat step 14 one more time.
8. Put the spin column back into the collection tube, centrifuge the empty tube at 12,000rpm (~13,400 ×g) for 2 minutes, and remove the residual Buffer WB in the spin column.
9. Move the spin column to a new 2ml centrifuge tube, drop **100μl** Buffer EB preheated at 65°C into the center of the membrane (do not add the eluent to the press ring, otherwise a large volume of eluate will be lost), place at room temperature for 5 min, and centrifuge at 12,000 rpm (~13,400 ×g) for 1 min. Drop **100μl** pre-warmed Buffer EB into the center of the membrane again and centrifuge at 12,000rpm (~13,400 ×g) for 1min. Combine the eluates from the two collections.

Note: If you want to increase the concentration of DNA, you can add the solution from the first centrifugation back to the spin column and centrifuge at 12,000rpm (~13,400×g) for 1min. In addition, when the sample volume is less than 100mg, the recommended elution volume is 60-100μl.