Ver. 23091

ODP311

# Stool DNA Kit

For extraction of stool DNA from mammalian stool sample

Origin®

ISO 13485:2016 ISO 9001:2015

# Stool DNA Kit

Cat No. ODP311

## Kit Contents

| Contents | 50 Preps |
|---|---|
| Buffer SL | 40mL |
| Buffer GL | 25mL |
| Buffer IR | 25mL |
| Buffer GD | 13mL |
| Buffer PW | 15mL |
| Buffer TE | 10mL |
| Proteinase K (20mg/mL) | 1.25mL |
| Spin column CB3 | 50 |
| Collection Tubes (2mL) | 50 |
| Handbook | 1 |

## Storage

Stool DNA Kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

**(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)**

Proteinase K should be stored at -20°C from receipt of the kit.

## Introduction

Stool DNA Kit is based on silica membrane technology and special buffer system for DNA extraction from stool samples. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants, inhibitors like humic acid and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Stool DNA Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

## Yield of Stool DNA

| Source (180mg/200µl) | Yield |
|---|---|
| Mammalian stool sample | 5-20µg |

## Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
2. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.
5. If a precipitate has formed in Buffer GB or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.
6. Repeated freezing and thawing of Proteinase K should be avoided; otherwise, it would reduce the DNA quality and quantity.

## Protocol

**Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.**

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1. Sample Preparation: for dry samples, weigh 180mg of stool sample in a 1.5ml micro-centrifuge tube. For liquid sample, pipette 200µL sample to the micro-centrifuge tube.
2. Add 25µL Proteinase-K to the sample and mix manually by inverting the tube.
3. Add 800µL Buffer SL to the mixture, mix manually and then incubate at 56˚C for 10 minutes.
4. Centrifuge at 12000rpm (~13,400 ×g) for 1 minute.
5. Transfer the supernatant to a 2mL tube, without disturbing the pellet.
6. Add 500µL Buffer GL to the supernatant and mix well by vortexing for 20 seconds. (Precipitates formed in this step will be dissolved after the incubation in next step)
7. Incubate at 95˚C for 5 minutes.
8. Add 500µL Buffer IR to the mixture and mix well by vortexing for 20 seconds. Incubate the tubes on ice for 5 minutes.
9. Centrifuge at 12,000 rpm (~13,400 ×g) for 5 minutes.
10. Transfer the supernatant to two new 1.5mL micro-centrifuge tubes (approximately 700µL in each tube) without disturbing the pellet.
11. Add 500µL (96-100%) ethanol to the supernatant in each of the tubes and votex for 20 seconds.

12. Pipette 750µL of the mixture from step 11 to a spin column CB3 (in a 2mL collection tube) and spin down briefly for 40 seconds. Then discard the flow-through and place the spin column CB3 to the collection tube.
13. Transfer the remaining mixture to spin column CB3 and repeat step 12 until the all the mixture is transferred to spin column CB3.
14. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
15. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
16. Add 500µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
17. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to dry the membrane completely.
    **Note: The resident ethanol of Buffer PW may have some affect in downstream application.**
18. Place the spin column CB3 in a new clean 1.5mL microcentrifuge tube, and pipette 50µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g).
    **Note: To increase the DNA yield,**
    - **Introduce the eluted Buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm**.
    - **Warm Buffer TE at 50-60°C before adding to the spin column CB3.**

    **If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.**