

## Kit Description

Stool sample contain many different kinds of pathogens and also brings cells or small tissue pieces from digestive tract. CatchGene™ Stool DNA Kit designed to purify all gDNA from stool samples include pathogen and human DNA. Homogenize stool properly is the first step and essential step to release gDNA from stool samples. Here we provide optimized beads which enhance homogenization efficiency of stool samples. Besides, stool samples are very complex mixture which contain many different kinds of inhibitors. The Buffer ST2 is a great solution to remove those inhibitors. With these combination of consumables and buffer system, CatchGene Stool DNA Kit is able to purify pure and complete gDNA from stool samples.

## Kit Content

	4 rxn	50 rxn	250 rxn	
Spin Column	4	50	250	pcs
Collection Tube (2 ml)	12	150	750	pcs
Beads Tube	4	50	250	tube
Proteinase K Solvent	0.5	1.5	10	ml
Proteinase K	1	11	11x5	mg
Buffer ST1	4.32	54	135x2	ml
Buffer ST2	1.44	18	90	ml
Buffer ST3	0.96	12	60	ml
Buffer W1 (concentrated)	3.36	42	105x2	ml
Buffer W2 (concentrated)	0.68	8.4	42	ml
Elution Buffer	0.96	12	60	ml

## Kit Storage

Upon arrival,  
 1. Please store **Proteinase K** at **-20 °C** for long term storage.

Buffer, solvent and consumables, please store at 15-25 °C .

If a precipitate has formed in Buffer ST1 or ST3, dissolve by incubating at 60°C for 10 min.

## Kit Preparation

- 1. Prepare 10 mg/ml Proteinase K**  
 For 1 mg Proteinase K, please add 100 µl Proteinase K Solvent into tube and vortex thoroughly for dissolving.  
 For 11 mg Proteinase K, please add 1100 µl Proteinase K Solvent solvent into tube and vortex thoroughly for dissolving.  
 After dissolving into solvent, please store in 4°C for 6 month or -20°C for 1 year.
- 2. Prepare Buffer W1**  
 Add equal volume of 100% EtOH into Buffer W1 (concentrated) to get Buffer W1.  
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.
- 3. Prepare Buffer W2**  
 Add 4 volume of 100% EtOH into Buffer W2 (concentrated) to get Buffer W2.  
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

## General Protocol

1. Weigh 180–220 mg stool in the Beads Tube and place the tube on ice.
2. Add 900 µl Buffer ST1 to each Bead Tube. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.
3. Heat the suspension for 15 min at 70°C. (If gram-positive bacteria are one of targeted pathogens, incubate at 95°C for 15 min is suggested.) Cool down to room temperature.
4. Add 300 µl Buffer ST2, vortex for 15 s and incubate on ice for 15 min.
5. Centrifuge sample at 12,000 x g at 4°C for 10 min to pellet stool debris.
6. Transfer 200 µl of the supernatant into a new 2 ml micro-centrifuge tube (not provided). (Avoid to aspirate any gel like precipitate or stool debris.)
7. Add 20 µl Proteinase K, vortex for 15 sec, brief spin down.
8. Add 200 µl Buffer ST3, vortex for 15 sec. Incubate at 70 °C for 10 min. Cool down to room temperature and brief spin down.
9. Add 200 µl 100% EtOH, vortex for 15 sec then brief spin down.
10. Transfer all lysate to the Spin Column (with 2ml Tube), centrifuge at 12,000 x g for 1 min.
11. Carefully move the Spin Column to a new Collection Tube (2 ml).
12. Add 700 µl Buffer W1 into the Spin Column, centrifuge at 12,000 x g for 1 min, discard the flow-through.
13. Repeat step 12 once.
14. Add 700 µl Buffer W2 into the Spin Column, centrifuge at 12,000 x g for 1 min, discard the flow-through.
15. Carefully move the Spin Column to a new Collection Tube (2 ml), centrifuge at 12,000 x g for 3 min.
16. Place the spin column into 1.5 ml micro-centrifuge tube (not provided), add 200 µl Elution Buffer and incubation at room temperature for 3 min, centrifuge at 12,000 x g for 1 min for elution.

**FOR RESEARCH USE ONLY**