CatchGene™ Microbiome DNA Kit

Cat. No.	Rxn
MD23004	4
MD23050	50
MD23250	250

Kit Content

	4rxn	50rxn	250rxn	
MD23 Column	4	50	250	pcs
Collection Tube (2 ml)	12	150	750	pcs
Beads Tube	4	50	250	tube
Buffer AE	0.5	1.5	10	ml
Proteinase K	1	11	11x5	mg
Carrier RNA	12	12x5	140x2	µg
Buffer DTL	2	25	125	ml
Buffer DL	1.8	22	110	ml
Buffer W1 (concentrated)	1.68	21	105	ml
Buffer W2 (concentrated)	0.68	8.4	42	ml
Elution Buffer	1	12	60	ml

Kit Storage

Upon arrival,

1. Please store **MD23 Column** at **4℃** for long term storage.
2. Please store **Proteinase K and carrier RNA** at **-20 ℃** for long term storage.

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

If any precipitate has formed in Buffer DTL or DL, 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 Buffer AE into tube and vortex thoroughly for dissolving.
   For 11 mg Proteinase K, please add 1100 µl Buffer AE solvent into tube and vortex thoroughly for dissolving.
   After dissolving into solvent, plase store in 4°C for 6 month or -20°C for 1 year.
2. **Prepare 0.5 µg/µl carrier RNA**
   For 12 µg Carrier RNA, please add 24 µl Buffer AE into the bottom of tube and mix thoroughly for dissolving.
   For 140 µg Carrier RNA, please add 280 µl Buffer AE into the bottom of tube and mix thoroughly for dissolving.
   After dissolving, please aliquot into smaller volume and store at -20°C. Do not freeze-thaw more than three times.
3. **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.
4. **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

For saliva, serum, plasma or other body fluid

1. Aliquot 1- 1.5 ml sample into a 1.5 ml micro-centrifuge tube.
2. Centrifuge sample at 11,000 x g for 3 min to pellet bacterium and fungi.
3. Remove most supernatant, leave 200 µl and do not disturb the pellet in the bottom.
4. Pipetting to suspend the pellet than transfer all sample into the Beads Tube.
5. Add 250 µl Buffer DTL into the Beads Tube vortex vigorously for 3 mins. Brief spin down.
6. (Optional) Incubate at 95°C for 15 min, then cool down to room temperature.
   *This step is for complete lysis of gram positive bacterium but might cause DNA degradation. If the integrity is very important for your following application, please skip this step.
7. Add 20 µl Proteinase K (10 mg/ml), vortex for 10 sec. Brief spin down, then incubate at 60 °C for 30 min.
8. Centrifuge sample at 11000 x g for 3 min, transfer 400 µl supernatant to a new 1.5ml micro-centrifuge tube.
9. (Optional) Add 4 µl RNase A (100mg/ml, not provided), then incubate at room temperature for 2 min.
10. Add 400 µl Buffer DL, vortex for 15 sec. Incubate at 70 °C for 15 min. Cool down to room temperature and brief spin down.
11. Add 400 µl 100% EtOH vortex for 15 sec then brief spin down.
12. Transfer 600 µl lysate to the MD23 Column (with 2ml Tube), centrifuge at 11,000 x g for 1 min, discard the flow-through.
13. Transfer all remaining lysate to the MD23 Column (with 2ml Tube), centrifuge at 11,000 x g for 1 min.
14. Carefully move the MD23 Column to a new Collection Tube (2 ml).
15. Add 700 µl Buffer W1 into the MD23 Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
16. Add 700 µl Buffer W2 into the MD23 Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
17. Add 700 µl 100% EtOH into the MD23 Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
18. Carefully move the MD23 Column to a new Collection Tube (2 ml), centrifuge at 13,000 x g for 3 min.
19. Place the MD23 Column into 1.5 ml micro-centrifuge tube, add 10-20 µl Elution Buffer and incubation at room temperature for 3 min, centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY

Sample Pretreatment

- **Dry paper point**
  1. Cut one paper point sample into around 3-5 mm length fragments and place into a beads tube.
  2. Add 450 µl Buffer DTL then vortex vigorously for 3 mins. Brief spin down.
  3. (Optional) Incubate at 95°C for 15 min, then cool down to room temperature.
     *This step is for complete lysis of gram positive bacterium but might cause DNA degradation. If the integrity is very important for your following application, please skip this step.
  4. Add 20 µl Proteinase K, vortex for 10 sec. Brief spin down, then incubate at 60°C for 60 min.
  5. Centrifuge sample at 11000 x g for 3 min, transfer 400 µl supernatant to a new 1.5 ml tube.
     *If the volume of supernatant is less than 400 µl, please compensate with Buffer DTL to 400 µl.
  6. Add 2 µl carrier RNA (0.5ug/ µl), vortex 5 sec for mixing.
  7. Proceed to **step 10 of General Protocol**.

- **Paper point preserved in 200 µl TE Buffer**
  1. Cut one paper point sample into around 3-5 mm length fragments and place into a beads tube.
  2. Transfer all preservation buffer with debris into the same bead tube.
  3. Add 250 µl Buffer DTL then vortex vigorously for 3 mins. Brief spin down.
  4. (Optional) Incubate at 95°C for 15 min, then cool down to room temperature.
     *This step is for complete lysis of gram positive bacterium but might cause DNA degradation. If the integrity is very important for your following application, please skip this step.
  5. Add 20 µl Proteinase K, vortex for 10 sec. Brief spin down, then incubate at 60°C for 60 min.
  6. Centrifuge sample at 11000 x g for 3 min, transfer 400 µl supernatant to a new 1.5ml tube.
     *If the volume of supernatant is less than 400 µl, please compensate with Buffer DTL 400 µl.
  7. Add 2 µl carrier RNA (0.5ug/ µl), vortex 5 sec for mixing.
  8. Proceed to **step 10 of General Protocol**.