CatchGene® *Catch-cfDNA* Saliva/Body fluid Kit

Cat. No.	Rxn
MC12002	2
MC12030	30

Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
LV Column	2	30	pcs
Collection Tube (2 ml)	4	60	pcs
Buffer AE	1.5	10	ml
Carrier RNA	12	180	µg
Proteinase K	10	150	mg
Buffer DCL	9.6	144	ml
Buffer CW1 (concentrated)	7.5	120	ml
Buffer CW2 (concentrated)	1.68	12	ml
Buffer EB	0.48	7.2	ml

Important Notice !
"LV Column" should be stored at 2–8°C upon arrival for long term storage**.**

Kit Preparation

1. **Prepare 20 mg/ml Proteinase K**
   For 10 mg Proteinase K, please add 0.5 ml Proteinase solvent into tube and vortex thoroughly for dissolving
   For 150 mg Proteinase K, please add 7.5 ml Proteinase solvet 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 AE Buffer into the bottom of tube and mix thoroughly for dissolving.
   For 180 µg Carrier RNA, please add 360 µl AE Buffer 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.
2. **Prepare Buffer CW1**
   Add equal volume of 100% EtOH into Buffer CW1 (concentrated) to get Buffer CW1.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.
3. **Prepare Buffer CW2**
   Add equal volume of 100% EtOH into Buffer CW2 (concentrated) to get Buffer CW2.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

Sample Pretreatment

The half life of cfDNA in saliva and body fluid is very short. So, after sampling please must perform pretreatment as soon as possible.

1. Centrifuge at 3,000 x g for 10 minute at room temperature.
2. Transfer upper layer to the 1.5 ml micro-centrifuge tube (not provided). Please avoid aspirating any cell pellet in the bottom of tube, otherwise will co-extract gDNA form intact cell
3. Centrifuge at 11,000 x g for 10 min and transfer the supernatant for following extraction.

Please keep samples into -20°C or -70°C if extraction won't be performed immidiately after pretreatment.

General Protocol

For **4 ml saliva/ body fluid sample**

1. Add 200 µl Proteinase K (20 mg/ml) into the bottom of 50 ml tube.
2. Add 10 µl Carrier RNA (0.5 µg/µl) into the 50 ml tube.
3. Transfer 4 ml of sample (already centrifuged with high speed) to 50 ml tube.
4. Add 4 ml Buffer DCL to 50 ml tube, vortex 30 sec.
5. 56℃ incubate for 30 min, then cool down to room temperature (25℃)
6. Add 4 ml 100% EtOH, vortex 15 sec.
7. Connect LV Module with LV Column to become LV Column Module. Please refer to the illustration in next page.
8. Transfer all lysate into LV Column Module, centrifuge at 2,700 x g for 2 min, discard the flow-through.
9. Add 7ml CW1 Buffer into LV Column Module, centrifuge at 2,700 x g for 2 min, discard the flow-through.
10. Take LV Column Module out of 50 ml tube. Disconnect the LV Column from the LV Module, then place the LV Column on a 2 ml Collection Tube. Please refer to the illustration in next page.
11. Add 700 µl CW2 Buffer into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
12. Repeat step 11 once.
13. Add 700 µl 100% EtOH into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
14. Place spin column on a new 2 ml Collection Tube, centrifuge at 11,000 x g for 3 min to eliminate any remaining EtOH.
15. Place spin column on a new 1.5 ml tube. Add 30-150 µl Buffer EB, incubation at room temperature for 5 min, and then centrifuge at 11,000 x g for 1 min for elution.

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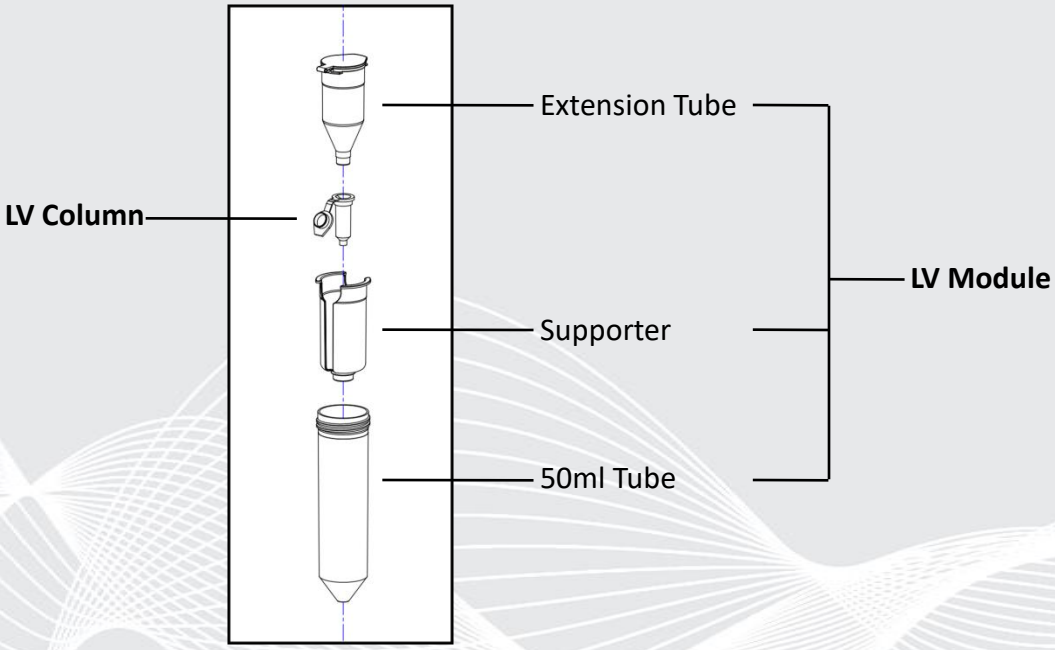
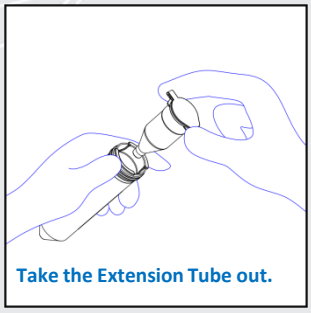
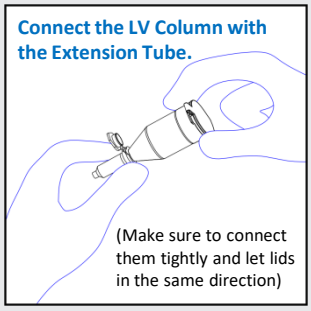
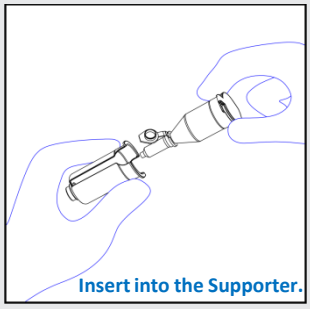
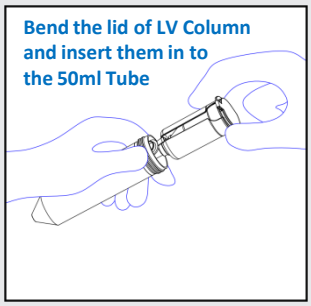


Connect the LV Module with the LV Column

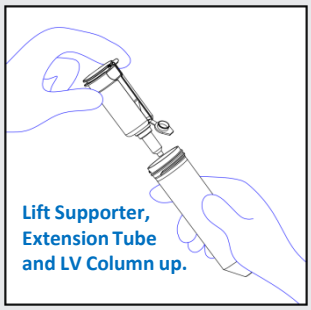


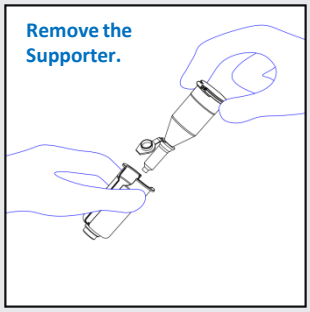


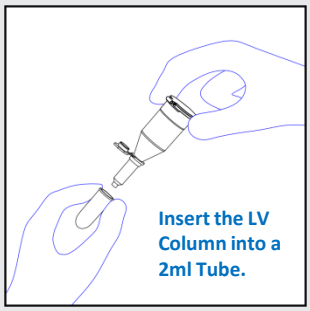


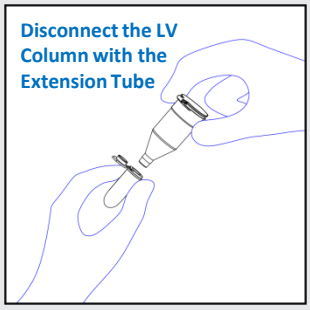


Disconnect the LV Column from the LV Column Module









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