CatchGene® *Catch-cfDNA* Urine Kit

Cat. No.	Rxn
MC11002	2
MC11030	30

Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
MC11 Column	2	30	pcs
Collection Tube (2 ml)	4	60	pcs
Buffer AE	1.5	10	ml
Carrier RNA	12	200	µg
Proteinase K	11	130	mg
Buffer DCL	21	165 x 2	ml
Buffer CW1 (concentrated)	7.5	120	ml
Buffer CW2 (concentrated)	1.68	12	ml
Elution Buffer	0.48	7.2	ml

Important Notice !
"**MC11 Column**" should be stored at 4°C upon arrival for long term storage**.**
"**Carrier RNA** and **Proteinase K**" should be stored at -20°C upon arrival for long term storage.

Kit Preparation

1. **Prepare 20 mg/ml Proteinase K**
   For 11 mg Proteinase K, please add 0.55 ml Buffer AE into tube and vortex thoroughly for dissolving
   For 130 mg Proteinase K, please add 6.5 ml Buffer AE 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 200 µg Carrier RNA, please add 400 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.
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

Please collect the first urination in the morning.
After sampling, please must perform pretreatment as soon as possible.
1. Centrifuge urine at 3,000 x g for 10 minute at room temperature.
2. Transfer upper clear layer to 50 ml tube (not provided). Please avoid aspirating any cell pellet, otherwise will co-extract gDNA from intact cell

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

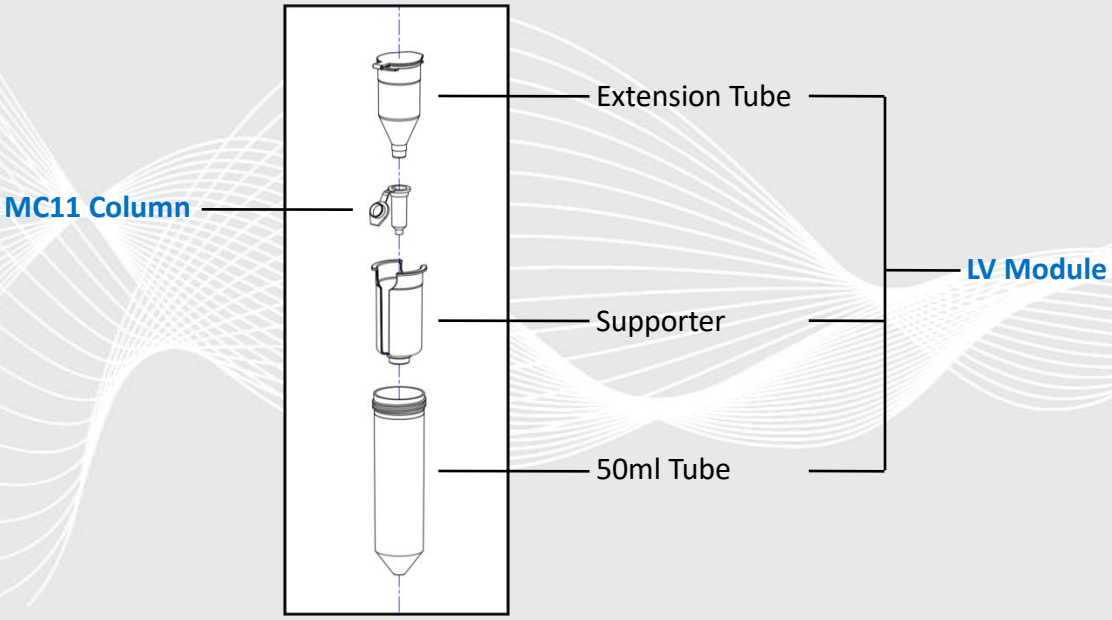
General Protocol

For **10 ml Urine 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 10 ml of urine sample into 50 ml tube, vortex 10 sec to let Proteinase K and samples mix thoroughly.
   Important! Avoid mixing Proteinase K with Buffer DCL directly. It will cause malfunction of Proteinase K and shortage of cfDNA. Please must mix Proteinase K with sample first, then add Buffer DCL .
4. Add 10 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 10 ml 100% EtOH, vortex 15 sec.
7. Connect LV Module with MC11 Column to become LV Column Module. Please refer to the illustration in next page.
8. Transfer 10 ml lysate into LV Column Module, centrifuge at 2,700 x g for 7 min, discard the flow-through. (If there is any remaining lysate above membrane, please centrifuge additional 2 min to let all lysate pass through the membrane.)
9. Repeat step 8 twice to let all lysate pass through the membrane.
10. Add 7ml CW1 Buffer into LV Column Module, centrifuge at 2,700 x g for 4 min, discard the flow-through. (If there is any remaining wash buffer above membrane, please centrifuge additional 2 min to let all lysate pass through the membrane.)
11. Take LV Column Module out of 50 ml tube. Disconnect the MC11 Column from the LV Module, then place the MC11 Column on a 2 ml Collection Tube. Please refer to the illustration in next page.
12. Add 700 µl CW2 Buffer into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
13. Add 700 µl CW2 Buffer into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
14. Add 700 µl 100% EtOH into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
15. Place spin column on a new 2 ml Collection Tube, centrifuge at 11,000 x g for 3 min to eliminate any remaining EtOH.
16. Place spin column on a new 1.5 ml micro-centrifuge tube. Add 10-20 µl Elution Buffer, incubation at room temperature for 5 min, and then centrifuge at 11,000 x g for 1 min for elution.

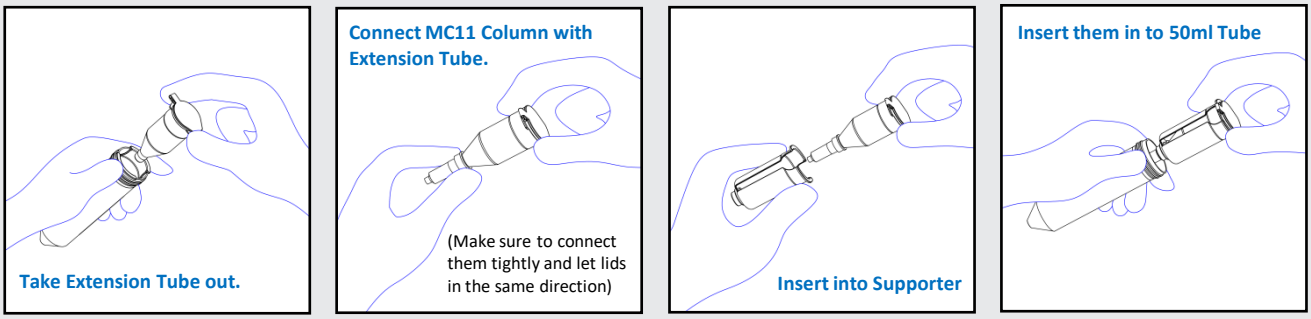
FOR RESEARCH USE ONLY

Kit Storage

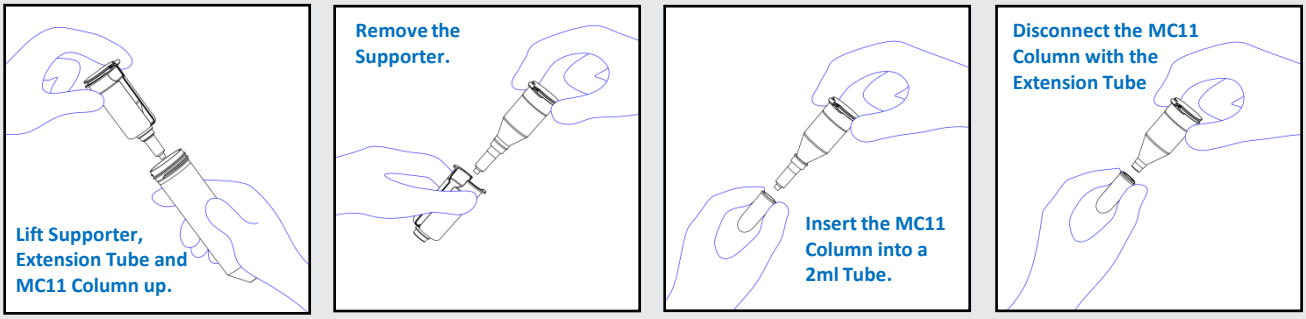
1. MC11 Column is able to ship at ambient temperature for 2 weeks. After receiving the kit, if you won't use them immediately, please take them out and store at 4°C for long term storage. (Do not freeze into -20 °C)
2. Proteinase K and Carrier RNA are lyophilized powder able to ship at ambient temperature for 2 weeks. After receiving the kit, if you won't use them immediately, please take them out and store at -20 °C for long term storage.
3. Buffer, solvent and consumables, please store at 15-25 °C.
   If a precipitate has formed in Buffer DCL, please dissolve by incubating at 60°C.



Connect LV Module with MC11 Column



Disconnect MC11 Column from LV Column Module



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