CatchGene® Ultra cfDNA/cfRNA Isolation Kit

Cat. No.	Rxn
MC31002	2
MC31030	30

Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
M Column	2	30	pcs
Collection Tube (2 ml)	4	60	pcs
Buffer AE	1.5	10	ml
Proteinase K	11	130	mg
Buffer TCL	9.6	144	ml
Buffer TW1 (concentrated)	13	185	ml
Elution Buffer	0.48	7.2	ml

Important Notice !
"**M Column**" should be stored at 4°C upon arrival for long term storage**.**
"**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, please store in 4°C for 6 month or -20°C for 1 year.
2. **Prepare Buffer TW1**
   Add equal volume of 100% EtOH into Buffer TW1 (concentrated) to get Buffer TW1.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

Sample Pretreatment

The half life of cfDNA/cfRNA in serum, plasma and body fluid is very short. So, after sampling by blood tube, please must perform pretreatment as soon as possible. For serum, plasma and body fluid sample

1. Centrifuge whole blood 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 debris or WBC, and intermediate layer, 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 ▲**1ml,** ●**2ml or** ■**4 ml** serum, plasma and body fluid sample

1. Add 50 µl (▲1ml) or 100 µl (●2ml) or 200 µl (■4 ml) Proteinase K (20 mg/ml) into the bottom of 50 ml tube (not provided).
2. Transfer ▲1ml, ●2ml or ■4 ml of sample (already centrifuged with high speed) to 50 ml tube, vortex 10 sec to let Proteinase K and samples mix thoroughly.
   Important! Avoid mixing Proteinase K with Buffer TCL directly. It will cause malfunction of Proteinase K and shortage of cfDNA/cfRNA. Please must mix Proteinase K with sample first, then add Buffer TCL .
3. Add 1 ml (▲1ml) or 2 ml (●2ml) or 4 ml (■4 ml) Buffer TCL to 50 ml tube, vortex 30 sec.
4. 56°C incubate for 30 min, then cool down to room temperature (25°C)
5. Add 1 ml (▲1ml) or 2.5 ml (●2ml) or 5 ml (■4 ml) 100% EtOH, vortex 15 sec.
6. Connect LV Module with M Column to become LV Column Module. Please refer to the illustration in next page.
7. Transfer all 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.)
8. (Optional) For maximum recovery rate, second binding is suggested. Please drain all lysate back to LV Column Module and perform step 7 again.
9. Add 3 ml (▲1ml) or 4.5 ml (●2ml) or 6 ml (■4 ml) TW1 Buffer into LV Column Module, centrifuge at 2,700 x g for 3 min, discard the flow-through.
10. Add 3 ml (▲1ml) or 4.5 ml (●2ml) or 6 ml (■4 ml) TW1 Buffer into LV Column Module, centrifuge at 2,700 x g for 3 min, discard the flow-through.
11. Add 3 ml (▲1ml) or 4.5 ml (●2ml) or 6 ml (■4 ml) 100% EtOH into LV Column Module, centrifuge at 2,700 x g for 3 min, discard the flow-through.
12. Add 3 ml (▲1ml) or 4.5 ml (●2ml) or 6 ml (■4 ml) 100% EtOH into LV Column Module, centrifuge at 2,700 x g for 3 min, discard the flow-through.
13. Disconnect the M Column from the LV Module. (Please refer to the illustration in next page.) Place the M Column on a 2 ml Collection Tube, centrifuge at 15,000 x g for 3 min to eliminate any remaining EtOH.
14. Place spin column on a new 2 ml Collection Tube, 56°C dry bath incubation for 10 min to evaporate any remaining EtOH.
15. 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.
16. (Optional) For higher concentration, repeated elution is suggested. Please aspirate the eluate and dispense back into the same column , incubation at room temperature for 5 min, and then centrifuge at 11,000 x g for 1 min for elution.
17. (Optional) For maximum yield, second elution is suggested. Please add another 10-20 µl Elution Buffer into column, and repeat step 15 again. Then mix 1st and 2nd eluate together.

FOR RESEARCH USE ONLY

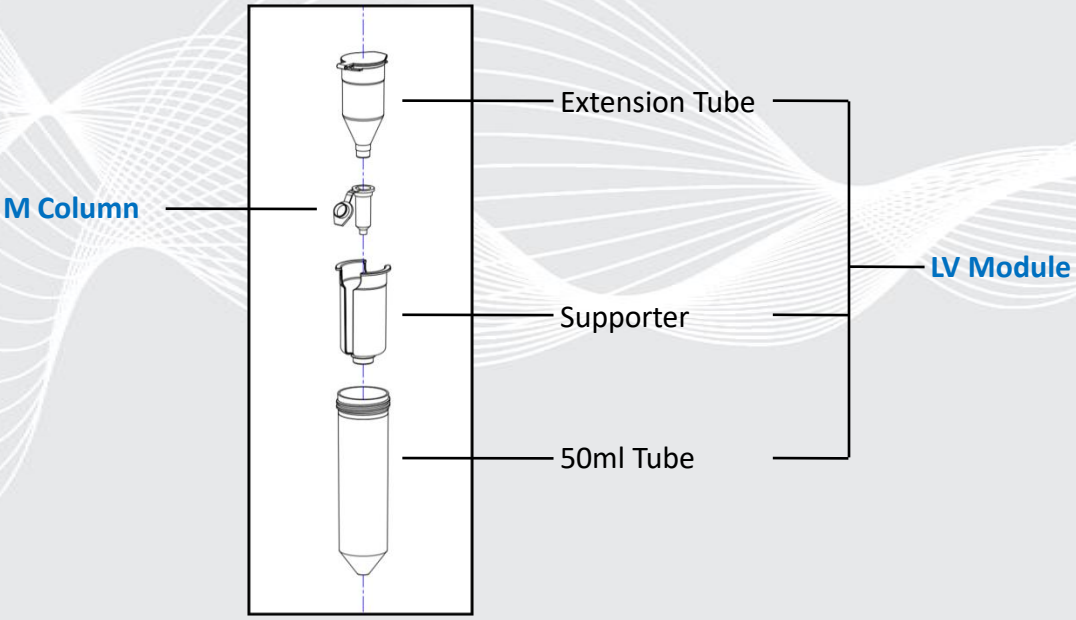
Ways to Thaw Sample

For plasma sample

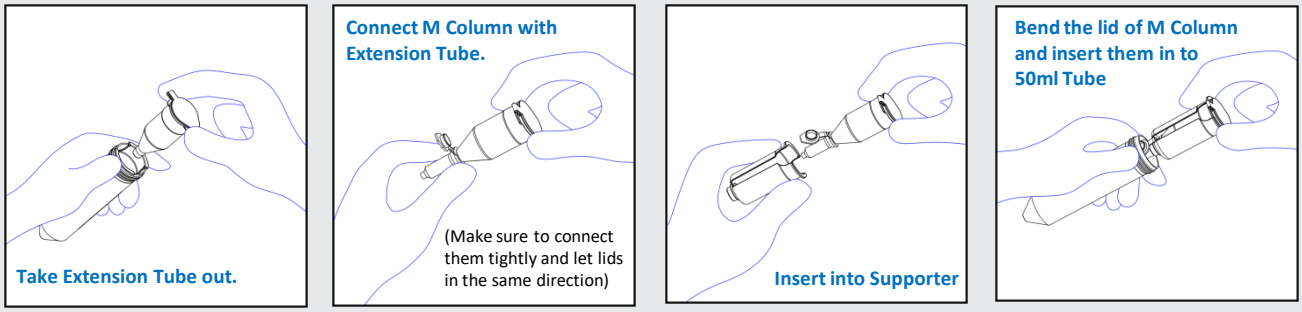
1. Please do not thaw samples on ice or at 4°C, it might cause the formation of cryoprecipitates.
2. Thaw samples at 30°C for 30 min is suggested to avoid the formation of cryoprecipitates.

Kit Storage

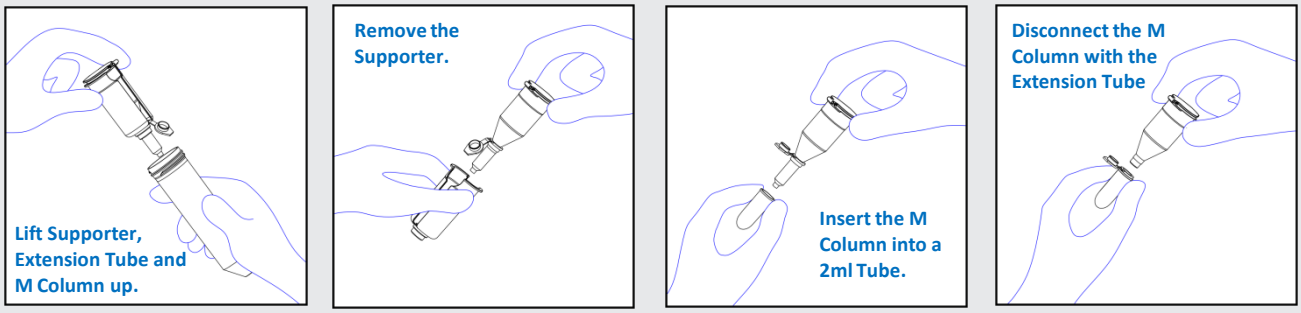
1. M Column is able to ship at ambient temperature for 2 weeks. After receiving the kit, if you won't use them immediately, please take them out and store at 4°C for long term storage. (Do not freeze into -20 °C)
2. Proteinase K is lyophilized powder able to ship at ambient temperature for 2 weeks. After 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 M Column



Disconnect M Column from LV Column Module



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