

## Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
LVR Column	2	30	pcs
Collection Tube (2 ml)	4	60	pcs
Buffer AE	1.5	10	ml
Carrier RNA	12	*164	µg
Proteinase K	11	130	mg
Buffer DBL	9.6	144	ml
Buffer CW1 (concentrated)	7.5	120	ml
Buffer CW2 (concentrated)	1.68	25	ml
Elution Buffer	0.48	7.2	ml

### Important Notice !

“LVR 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.

\*164 µg are the amount of two tubes of 12 µg Carrier RNA with one tube of 140 µg Carrier RNA.

## 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 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.

### 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 immediately after pretreatment.

## General Protocol

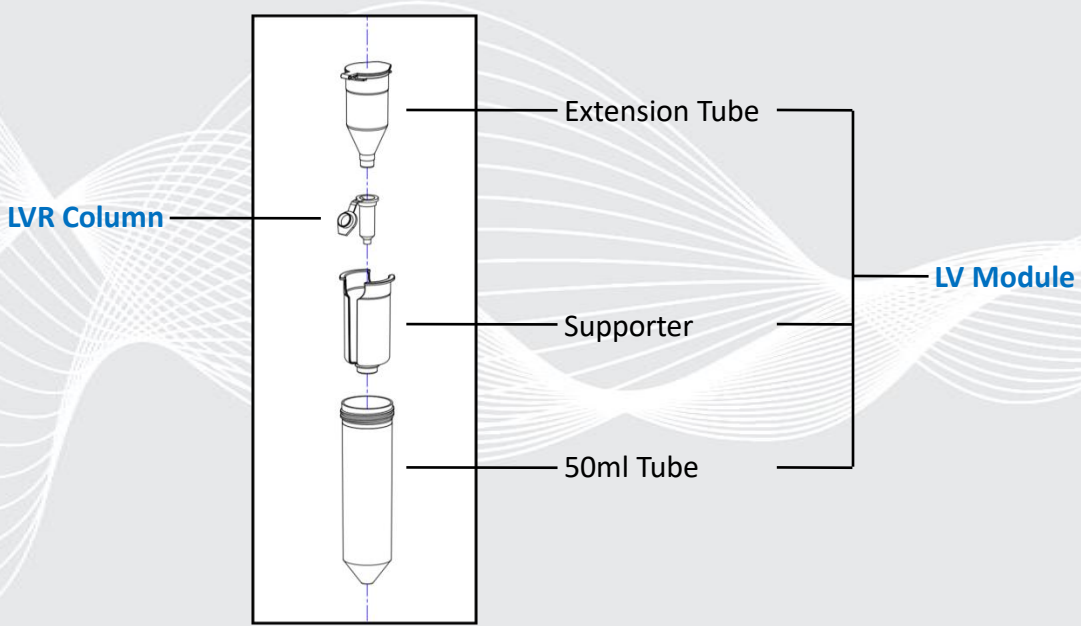
### For 4 ml Saliva/Body fluid sample

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.  
*Important! Avoid mixing Proteinase K with Buffer DBL directly. It will cause malfunction of Proteinase K and shortage of cfDNA. Please must mix Proteinase K with sample first, then add Buffer DBL .*
4. Add 4 ml Buffer DBL to 50 ml tube, vortex 30 sec.
5. 56°C incubate for 30 min, then cool down to room temperature (25°C)
6. Add 4 ml 100% EtOH, vortex 15 sec.
7. Connect LV Module with LVR 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 LVR Column from the LV Module, then place the LVR 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 micro-centrifuge tube. Add 30-150 µl Elution Buffer, incubation at room temperature for 5 min, and then centrifuge at 11,000 x g for 1 min for elution.

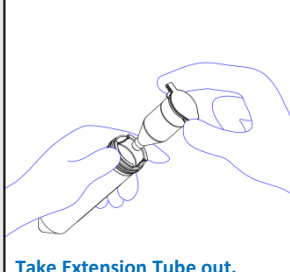
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## Kit Storage

1. LVR 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 DBL, please dissolve by incubating at 60°C.

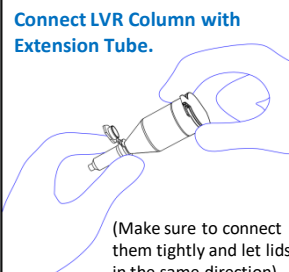


### Connect LV Module with LVR Column

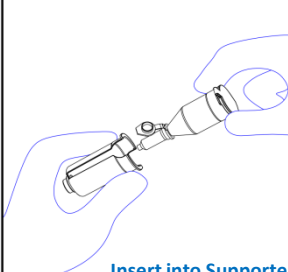


**Take Extension Tube out.**

**Connect LVR Column with Extension Tube.**

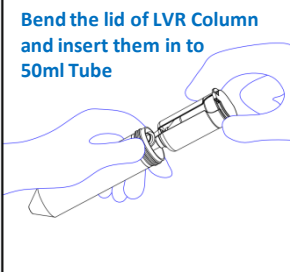


(Make sure to connect them tightly and let lids in the same direction)

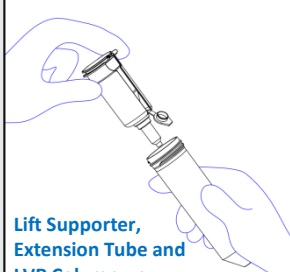


**Insert into Supporter**

**Bend the lid of LVR Column and insert them in to 50ml Tube**




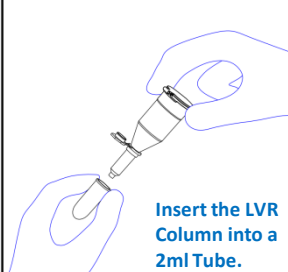
### Disconnect LVR Column from LV Column Module



**Lift Supporter, Extension Tube and LVR Column up.**

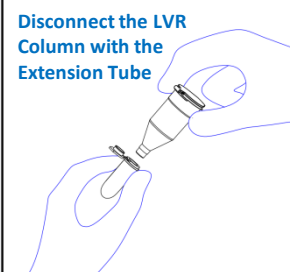
**Remove the Supporter.**





**Insert the LVR Column into a 2ml Tube.**

**Disconnect the LVR Column with the Extension Tube**



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