

## Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
LV B 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	21	165 x 2	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 B 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 Buffer AE into tube and vortex thoroughly for dissolving  
 For 150 mg Proteinase K, please add 7.5 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 180 µg Carrier RNA, please add 360 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

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

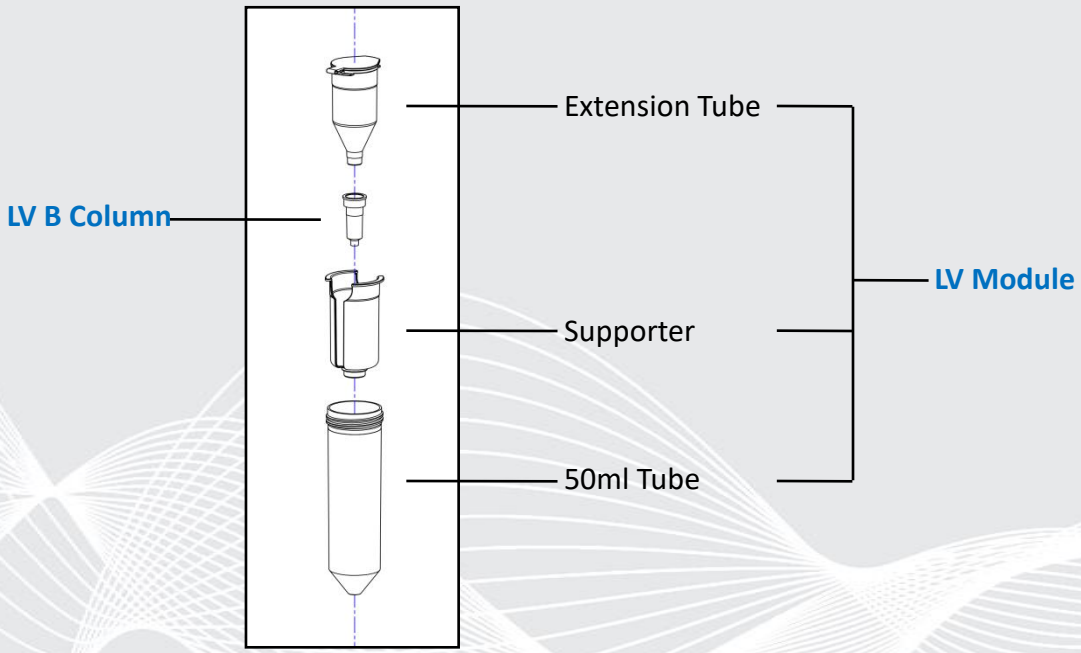
Please keep samples into -20°C or -70°C if extraction won't be performed immediately after pretreatment.

## General Protocol

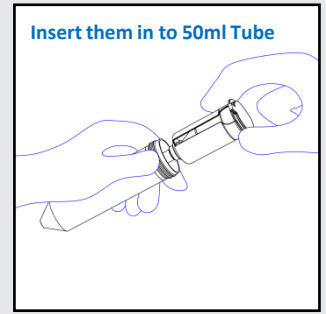
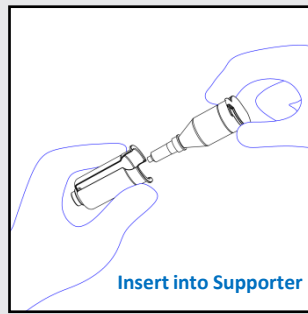
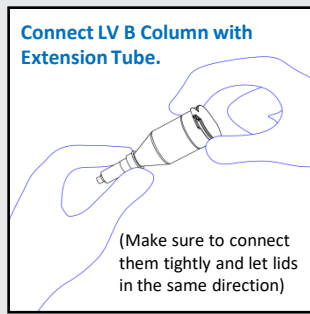
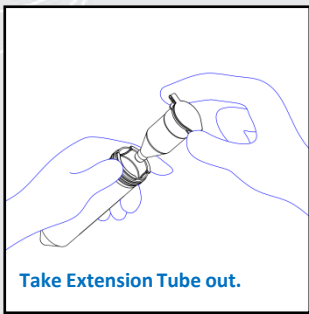
### For 10 ml Urine sample

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 sample to 50 ml tube.
4. Add 10 ml Buffer DCL to 50 ml tube, vortex 30 sec.
5. 56°C incubate for 30 min, then cool down to room temperature (25°C)
6. Add 10 ml 100% EtOH, vortex 15 sec.
7. Connect LV Module with LV B 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 5 min, discard the flow-through.
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 2 min, discard the flow-through.
11. Take LV Column Module out of 50 ml tube. Disconnect the LV Column from the LV Module, then place the LVR 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. Repeat step 11 once.
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 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.

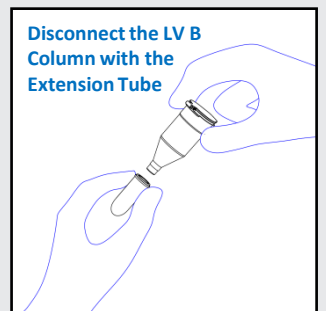
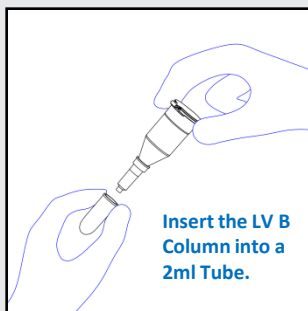
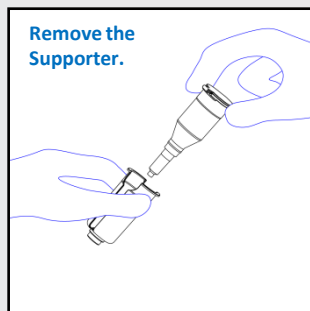
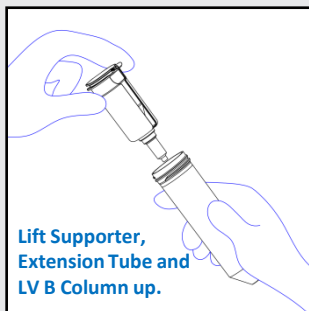
**FOR RESEARCH USE ONLY**



### Connect LV Module with LV B Column



### Disconnect LV B Column from LV Column Module



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