

## Kit Description

The Oil DNA Kit is designed to purify very rare DNA from large volume of oil sample. Based on optimized reagent buffer and silica membrane column, we are able to recovery most DNA inside of large volume oil sample which can be used in wide range of downstream application such as qPCR, Microarray and NGS.

## Kit Content

	2rxn	30rxn	
LV Module (with 50ml tube)	2	30	set
MD24 Column	2	30	pcs
Collection Tube (2 ml)	4	60	pcs
Plastic Dropper	2	30	pcs
Buffer AE	0.5	3	ml
Carrier RNA	12	*164	µg
Proteinase K	1x2	11x3	mg
Buffer O1	13	200	ml
Buffer O2	9	135	ml
Buffer CW1 (concentrate)	7.5	120	ml
Buffer CW2 (concentrate)	1.68	25	ml
Elution Buffer	0.48	7.2	ml

### Important Notice !

“MD24 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 1 mg Proteinase K, please add 50 µl Buffer AE into tube and vortex thoroughly for dissolving  
 For 11 mg Proteinase K, please add 550 µl 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 (concentrate) 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 (concentrate) to get Buffer CW2.  
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

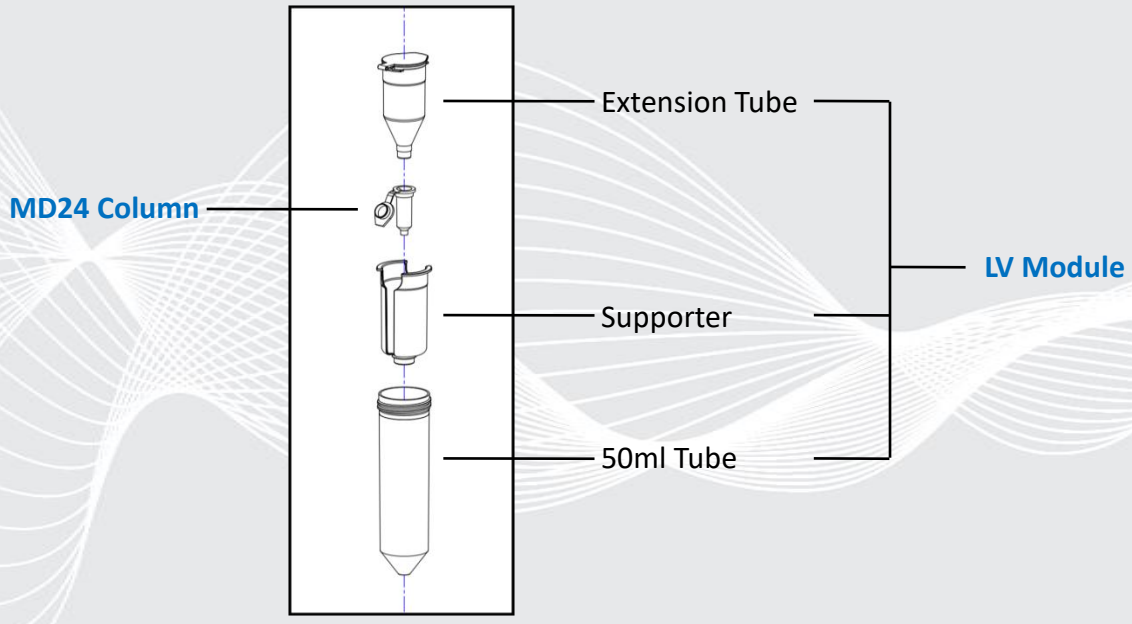
## General Protocol

For **40 ml Oil sample**

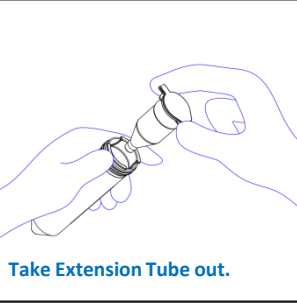
1. Add 6 ml Buffer O1 into a 50 ml tube (not provided) .
2. Transfer 40 ml oil sample into the 50 ml tube, vortex vigorously for 2 min.
3. 95°C incubate for 15 min, then cool down to room temperature (25°C).
4. Centrifuge at 2,600 x g for 10 min.
5. Use the plastic dropper to aspirate all (around 5 ml) lower clear phase into a new 50 ml tube (not provided).
6. Centrifuge at 2,600 x g for 5 min.
7. Pipette 4 ml lower clear phase lysate (avoid aspirating any oil in upper layer) into a new 50 ml tube.
8. Add 50 µl Proteinase K (20 mg/ml) and 10 µl Carrier RNA (0.5 µg/µl) into the 50 ml tube, vortex mix for 5 sec.
9. Add 4 ml Buffer O2 to the 50 ml tube, vortex vigorously for 30 sec.
10. 56°C incubate for 30 min, then cool down to room temperature (25°C)
11. Add 4 ml 100% EtOH, vortex mix for 15 sec.
12. Connect LV Module with MD24 Column to become LV Column Module. Please refer to the illustration in next page.
13. Transfer all lysate into LV Column Module, centrifuge at 2,700 x g for 2 min, discard the flow-through.
14. Add 7ml CW1 Buffer into LV Column Module, centrifuge at 2,700 x g for 2 min, discard the flow-through.
15. Take LV Column Module out of 50 ml tube. Disconnect the MD24 Column from the LV Module, then place the MD24 Column on a 2 ml Collection Tube. Please refer to the illustration in next page.

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16. Add 700  $\mu$ l CW2 Buffer into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
17. Add 700  $\mu$ l CW2 Buffer into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
18. Add 700  $\mu$ l 100% EtOH into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
19. Place spin column on a new 2 ml Collection Tube, centrifuge at 11,000 x g for 3 min to eliminate any remaining EtOH.
20. Place spin column on a new 1.5 ml micro-centrifuge tube (not provided). Add 30-150  $\mu$ l Elution Buffer, incubation at room temperature for 5 min, and then centrifuge at 11,000 x g for 1 min for elution.

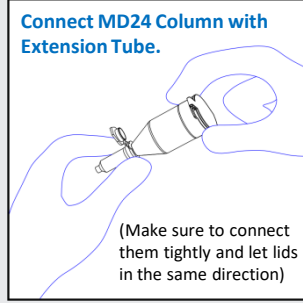


### Connect LV Module with MD24 Column

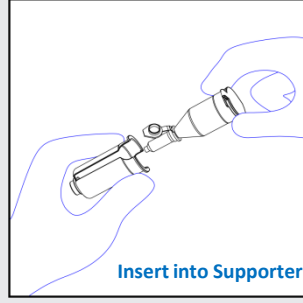


**Take Extension Tube out.**

**Connect MD24 Column with Extension Tube.**

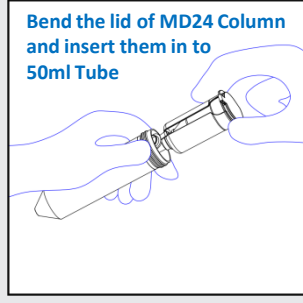


(Make sure to connect them tightly and let lids in the same direction)

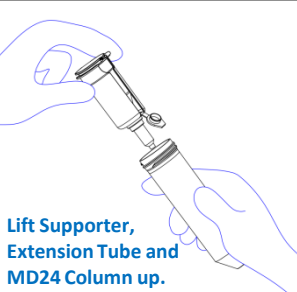


**Insert into Supporter**

**Bend the lid of MD24 Column and insert them in to 50ml Tube**

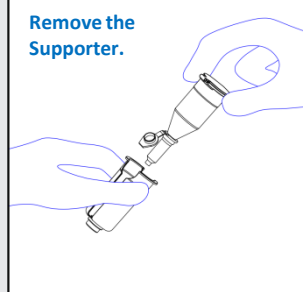


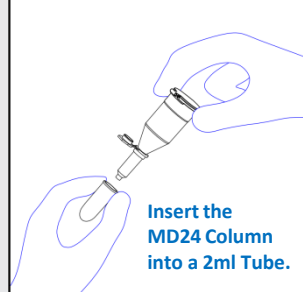
### Disconnect MD24 Column from LV Column Module



**Lift Supporter, Extension Tube and MD24 Column up.**

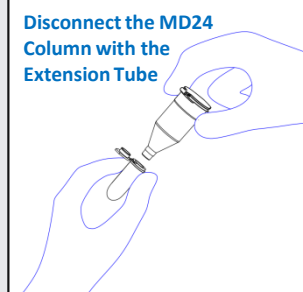
**Remove the Supporter.**





**Insert the MD24 Column into a 2ml Tube.**

**Disconnect the MD24 Column with the Extension Tube**



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