

## Kit Content

	4rxn	50rxn	250rxn	
Spin Column	4	50	250	pcs
Collection Tubes (2 ml)	12	150	750	pcs
Buffer AE	0.5	1.5	10	ml
Carrier RNA	12x2	140x2	1350	µg
Proteinase K	1	11	55	mg
Buffer TVL	1	11	55	ml
Buffer RW1 (concentrated)	1.6	20	100	ml
Buffer RW2 (concentrated)	0.7	8	40	ml
RNase-Free H <sub>2</sub> O	1	10	50	ml

## Kit Storage

Upon arrival,

1. Please store **Proteinase K** and **Carrier RNA** at **-20 °C** for long term storage.

Buffer, solvent and consumables, please store at 15-25 °C .

## Kit Preparation

### 1. Prepare 10 mg/ml Proteinase K

For 1 mg Proteinase K, please add 100 µl Buffer AE into tube and vortex thoroughly for dissolving.  
 For 11 mg Proteinase K, please add 1100 µl Buffer AE into tube and vortex thoroughly for dissolving.  
 For 55 mg Proteinase K, please add 5.5 ml Buffer AE into tube and vortex thoroughly for dissolving.  
 After dissolving into the solvent, please store at 4°C for 6 month or -20°C for 1 year.

### 2. Prepare 1 µg/µl Carrier RNA

For 12 µg Carrier RNA, please add 12 µl Buffer AE into the bottom of tube and mix thoroughly for dissolving.  
 For 140 µg Carrier RNA, please add 140 µl Buffer AE into the bottom of tube and mix thoroughly for dissolving.  
 For 1350 µg Carrier RNA, please add 1350 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.

### 3. Prepare Buffer RW1

Add equal volume of 100% EtOH into Buffer RW1 (concentrated) to get Buffer RW1.  
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

### 4. Prepare Buffer RW2

Add 4 volume of 100% EtOH into Buffer RW2 (concentrated) to get Buffer RW2.  
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

## Sample Pretreatment

For whole blood

1. Centrifuge whole blood at 3,000 x g for 10 minute at room temperature.
2. Transfer upper serum or plasma layer for following purification.

For nasopharyngeal swab with transport medium

1. Close the cap properly and vortex for 15 sec.
2. Transfer 200 µl clear supernatant for following purification. (Avoid to aspirate any debris or mucus)

## General Protocol

1. Add 20 µl Proteinase K (10 mg/ml) into a 1.5 ml micro-centrifuge tube (not provided).
2. Add 5 µl Carrier RNA (1 µg/µl) into the 1.5 ml micro-centrifuge tube.
3. Transfer 200 µl liquid sample into the tube, pipette thoroughly to mix sample with Proteinase and Carrier RNA .  
*Important! Avoid mixing Proteinase K with Buffer TVL directly. It will cause malfunction of Proteinase K. Please must mix Proteinase K with sample first, then add Buffer TVL .*
4. Add 200 µl Buffer TVL into the tube. Close the cap, vortex vigorously for 15 sec then brief spin down.
5. Incubate at 56 °C for 15 min, then cool down to room temperature.  
 (If RNA virus is the only target, incubate at 25 °C for 10 min is enough for proper lysis.)
6. Add 250 µl of 100% EtOH, close the cap and mix thoroughly by vortex for 15 sec, brief spin down.
7. Transfer all mixture to a Spin Column (with 2ml Tube), centrifuge at 11,000 x g for 1 min. Discard the flow-through and change a new Collection Tube.
8. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
9. Add 700 µl Buffer RW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
10. Add 700 µl 100% EtOH into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
11. Change a new Collection Tube, centrifuge at 11,000 x g for 3 min.
12. Place the spin column into a 1.5 ml micro-centrifuge tube, add 30-100 µl RNase-Free H<sub>2</sub>O and incubate at 25°C (room temperature) for 3 min.
13. Centrifuge at 11,000 x g for 1 min for elution.