

Kit Content

	4 rxn	50 rxn	
MD25 Column	4	50	pcs
Collection Tube (2 ml)	12	150	pcs
Buffer AE	1	2	ml
Carrier RNA	12	140	µg
Proteinase K	1	11	mg
Buffer F1*	1.9	24	ml
Buffer F2*	1.5	15	ml
Buffer W1 (concentrated)	1.68	21	ml
Buffer W2 (concentrated)	0.68	8.4	ml
Elution Buffer	0.96	12	ml

Kit Storage

- Upon arrival,
- Please store **MD25 Column** at **4°C** for long term storage.
 - Please store **Proteinase K** at **-20°C** for long term storage.

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

*There might precipitate in Buffer F1 or Buffer F2 if stored at low temperature. Dissolve it by incubation the buffer at 60°C for 10~20 mins.

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
 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.

3. Prepare Buffer W1

Add equal volume of 100% EtOH into Buffer W1 (concentrated) to get Buffer W1.
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

4. Prepare Buffer W2

Add 4 volume of 100% EtOH into Buffer W2 (concentrated) to get Buffer W2.
 After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

General Protocol

- Place sample into a 1.5ml micro-centrifuge tube (not provided).
- Add 130 µl Buffer F1.
- Add 20 µl Proteinase K and mix by vortex for 10 sec. Brief spin down.
- Incubate at 60 °C for 1 hour (with 900 rpm shaking or manual vortex for 10 sec every 10 min) then brief spin down.
- Add 150 µl Buffer F2 and mix by vortex for 10 sec. Brief spin down.
- (Optional) Add 2 µl Carrier RNA (0.5 µg/µl) into the 1.5 ml micro-centrifuge tube.
- Incubate at 70 °C for 10 min (with 900 rpm shaking or manual vortex for 10 sec every 3 min) then brief spin down.
- Cool down to room temperature and brief spin down.
- Add 150 µl 100% EtOH and mix thoroughly by pulse-vortex for 15 sec then brief spin down.
- Transfer all lysate to the MD25 Column (with 2ml Tube), centrifuge at 11,000 x g for 1 min.
- Carefully move the MD25 Column to a new Collection Tube (2 ml).
- Add 500 µl Buffer W1 into the MD25 Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
- Add 500 µl Buffer W2 into the MD25 Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
- Add 500 µl 100% EtOH into the MD25 column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
- Carefully move the Spin Column to a new Collection Tube (2 ml), centrifuge at 13,000 x g for 3 min.
- Place the spin column into 1.5 ml micro-centrifuge tube, add 10-20 µl Elution Buffer and incubation at room temperature for 3 min, centrifuge at 11,000 x g for 1 min for elution. (For highest recovery, second elution with another 10-20 µl Elution Buffer is possible.)

FOR RESEARCH USE ONLY