

Kit Content

	4rxn	50rxn	250rxn	
Spin Columns	4	50	250	pcs
Collection Tubes (2 ml)	12	150	750	pcs
Buffer RTL	2.4	30	150	ml
Buffer RB	0.5	6.3	31.5	ml
Buffer RW1 (concentrated)	3.36	42	105x2	ml
Buffer RW2 (concentrated)	0.68	8.4	42	ml
RNase-Free H ₂ O	0.96	12	60	ml

Kit Storage

Columns, buffers, solvents and consumables, please store at 15-25 °C.

Kit Preparation

1. Prepare Buffer RB

Add 2.4 volume of 100% EtOH into Buffer RB and vortex thoroughly.
After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.

2. 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.

3. 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.

General Protocol

For cell RNA extraction

- To collected the cells depend on cells growing type (cell number recommended not more than 1×10^7):
 - Harvested the cells grown in suspension by centrifuging for 5 min at 300 x g. Carefully remove supernatant and lysis cells with 350 μ l Buffer RTL (add 1% β -mercaptoethanol freshly), vortex vigorously for 30 sec, brief spin down then incubate at 25°C (room temperature) for 5 min. Proceed with step 4.
 - Cells grown in a monolayer should trypanized and centrifuge as a pellet remove the supernatant. add 350 μ l Buffer RTL (add 1% β -mercaptoethanol freshly), vortex vigorously for 30 sec, brief spin down then incubate at 25°C (room temperature) for 5 min. Proceed with step 4.

For tissue RNA extraction

- Weight up to 25 mg of animal tissue or no more than 10 mg spleen tissue.
 - Homogenize tissue sample with liquid nitrogen.
Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle. Proceed with step 2.
- Add 450 μ l Buffer RTL (add 1% β -mercaptoethanol freshly), vortex vigorously for 30 sec, brief spin down then incubate at 25°C (room temperature) for 5 min.
- Centrifuge at 11,000 x g for 3 min. Transfer 350 μ l of clear supernatant to a new 1.5 ml micro-centrifuge tube.
- Add 350 μ l Buffer RB, vortex for 30 sec then briefly spin down.
- Transfer all mixture to Spin Column (with 2ml Tube).
- Centrifuge at 11,000 x g for 1 min, and change a new collection tube.
- Add 700 μ l Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
- Repeat step 7 once.
- Add 700 μ l Buffer RW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
- Change a new collection tube, centrifuge at 11,000 x g for 3 min.
- Place the Spin Column into 1.5 ml micro-centrifuge tube, add 30-100 μ l RNase-Free H₂O and incubate at 25°C (room temperature) for 2 min.
- Centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY



Sample Pretreatment

- **PAXgene Blood RNA Tube**

1. Centrifuge the PAXgene Tube at 3,000 – 5,000 x g for 10 min. (Make sure the blood sample has been incubated in the tube for at least 2 hours at room temperature.)
2. Carefully drain or aspirate the supernatant away and avoid disturbing the pellet.
3. Add 4 ml RNase-free water into the tube and suspend the pellet by vortex gently.
4. Centrifuge the PAXgene Tube at 3,000 – 5,000 x g for 10 min.
5. Remove the supernatant thoroughly by pipette without disturbing the pellet.
6. Add 450 µl Buffer RTL (add 1% β-mercaptoethanol freshly) into the bottom of the PAXgene Tube, suspend the pellet by pipetting then transfer all lysis buffer and cells into a new 1.5ml tube (not provided).
7. Close the cap tightly and vortex vigorously for 30 sec, brief spin down then incubate at 25°C (room temperature) for 5 min.
8. Proceed to **step 3 of General Protocol**.

FOR RESEARCH USE ONLY