CatchGene® Cell/Tissue RNA Kit

Cat. No.	Rxn
MR20004	4
MR20050	50
MR20250	250

Kit Content

	4rxn	50rxn	250rxn	
Spin Columns	4	50	250	pcs
Collection Tubes (2 ml)	12	150	750	pcs
Buffer RTL	2	25	125	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_2O	0.96	12	60	ml

Kit Storage

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

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

1. To collected the cells depend on cells growing type (cell number recommended not more than 1 x 10^7):
   A. Harvested the cells grown in suspension by centrifuging for 5 min at 300 x g. Carefully remove supernatant and lysis cells with 350 µl 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.
   B. Cells grown in a monolayer should trypnized and centrifuge as a pellet remove the supernatant. add 350 µl 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

1. Weight up to 25 mg of animal tissue or no more than 10 mg spleen tissue.
   A. 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.
2. Add 450 µl 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.
3. Centrifuge at 11,000 x g for 3 min. Transfer 350 µl of clear supernatant to a new 1.5 ml micro-centrifuge tube.
4. Add 350 µl Buffer RB, vortex for 30 sec then briefly spin down.
5. Transfer all mixture to Spin Column (with 2ml Tube).
6. Centrifuge at 11,000 x g for 1 min, and change a new collection tube.
7. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
8. (Optional) On column digest of DNA with DNase I (not provided).
9. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
10. Add 700 µl Buffer RW2 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 1.5 ml micro-centrifuge tube, add 30-100 µl RNase-Free H_2O and incubate at 25°C (room temperature) for 2 min.
13. Centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY

Sample Pretreatment

- **For 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 pipettemen 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 Yeast**
  1. Transfer 1 ml log-phase (O.D.600=1) yeast culture to a 2ml Sample Tube. Do not load more than 2 x 10^7 cells. Overloading might cause bad in yield and purity.
  2. Descend the yeast cells by centrifuging at 1,000 x *g* for 5 min at 4℃ and discard the supernatant completely.
  3. Please carefully remove any remaining media by aspiration. Remaining media will affect digestion of the cell wall.
  4. Resuspend the cell pellet in100 µl sorbitol buffer prepared by RNase free water (1M sorbitol; 100 mM EDTA). (Please add 1%β-ME into the sorbitol buffer freshly.)
  5. Add 20-200 U zymolase or lyticase, incubate at 30℃ for 10-30 minute.
  6. Centrifuge at 500 x g for 5 min and discard the supernatant completely.
  7. Proceed to step 2 of Tissue RNA Extraction protocol.