CatchGene® FFPE Tissue miRNA Kit

Cat. No.	Rxn
MR23004	4
MR23050	50
MR23250	250

Kit Content

	4rxn	50rxn	250rxn	
MR23 Column	4	50	250	pcs
Collection Tube (2 ml)	12	150	750	pcs
Buffer AE	0.5	1.5	10	ml
Proteinase K	1x2	11x2	11x10	ml
Buffer DWX	2	27	135	ml
Buffer RFTL	0.7	9	45	ml
Buffer RFB	0.7	9	45	ml
Buffer RCL1	0.36	4.5	22.5	ml
Buffer RCL2	0.12	1.5	7.5	ml
Buffer CRW1 (concentrate)	0.65	8.5	42	ml
Buffer CRW2 (concentrate)	1.3	17	42x2	ml
RNase-Free H_2O	0.96	12	60	ml

Kit Storage

Upon arrival,

1. Please store **MR23 Column** at **4℃** for long term storage.
2. Please store **Proteinase K** at **-20 ℃** for long term storage.

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

If a precipitate has formed in Buffer RFTL or RFB, dissolve by incubating at 60°C and cool down to 25°C for using.

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 the solvent, please store at 4°C for 6 month or -20°C for 1 year.
1. **Prepare Buffer CRW1**
   Add 4 volume of 100% EtOH into concentrated Buffer CRW1 to get Buffer CRW1.
   After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.
2. **Prepare Buffer CRW2**
   Add 4 volume of 100% EtOH into concentrated Buffer CRW2 to get Buffer CRW2.
   After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.

General Protocol

1. Place 5-10 µm sections (up to 4 sections) in the micro-centrifuge tube (not provided). Add 450 µl DWX buffer, vortex vigorously for 15 sec. Spin down to collect sample in the bottom.
2. Incubate at 60℃ for 5 min. Brief spin down.
3. Add 150 µl Buffer RFTL (Please add 1% ß- mercaptoethanol freshly) and mix thoroughly by vortex 15 sec.
4. Centrifuge at 11,000 x g for 1 min.
5. Add 20 µl Proteinase K (20 mg/ml) to the lower clear phase. Mix gently by pipetting.
6. Incubate at 60℃ for 15 min. Brief spin down.
7. Incubate at 80℃ for 15 min.
8. Add 150 µl Buffer RFB in to the lower phase, mix gently by pipetting. Centrifuge at 11,000 x g for 1 min.
9. Aspirate 250 µl lower clear phase lysate into a new 1.5 ml micro-centrifuge tube.
2. Add 75 µl Buffer RCL1. Pulse-vortexing for 10 sec , brief spin down then incubate at 25°C (room temperature) for 3 min.
3. Add 25 µl Buffer RCL2, pulse-vortexing for 10 sec, brief spin down then incubate at 25°C (room temperature) for 1 min.
4. Centrifuge at 11,000 x g for 3 min.
5. Transfer 250 µl clear supernatant to a new 1.5 ml micro-centrifuge tube, add 330 µl of isopropanol, pulse-vortexing for 10 sec then briefly spin down.
6. Transfer all mixture to MR23 Column (with 2ml Collection Tube), incubate at 25°C (room temperature) for 2 min.
7. Centrifuge at 11,000 x g for 1 min. Change a new collection tube.
8. Add 700 µl Buffer CRW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
9. (Optional) On column digest of DNA with DNase I (not provided).
10. Add 700 µl Buffer CRW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
11. Add 700 µl Buffer CRW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
12. Change a new collection tube, centrifuge at 11,000 x g for 3 min.
13. 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.
14. Centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY