

Kit Description

The CatchGene™ FFPE Tissue RNA kit provides a safer, quicker and more efficient silica membrane-based RNA purification from Formalin-fixed, Paraffin-embedded FFPE tissue sample. Traditional method in pre-treatment of FFPE tissue sample uses xylene, which is highly toxic and carcinogenic. Hence, CatchGene FFPE Tissue RNA Kit offers a safer and optimized xylene-free method for RNA purification from FFPE tissue. Furthermore, Buffer RFTL and RFB is able to lyse FFPE tissue efficiently, and eventually minimize vortex steps to prevent fragmentation and preserve high integrity of RNA.

Kit Content

	4rxn	50rxn	250rxn	
MR21 Column	4	50	250	pcs
Collection Tubes (2 ml)	12	150	750	pcs
Buffer AE	0.5	1.5	10	ml
Proteinase K	1x2	11x2	11x10	mg
Buffer DWX	2	27	135	ml
Buffer RFTL	1.1	13.5	67.5	ml
Buffer RFB	1.1	13.5	67.5	ml
Buffer RW1 (concentrated)	3.36	42	105x2	ml
Buffer RW2 (concentrated)	0.68	8.4	42	ml
RNase-Free H₂O	0.96	12	60	ml

Kit Storage

Upon arrival,

1. Please store **MR21 Column** at 4°C for long term storage.
2. Please store **Proteinase K** at -20°C for long term storage.

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

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.
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.

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°C for 20 min. Brief spin down.
3. Add 225 µl Buffer RFTL (Please add 1% β- 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°C for 30 min. (Or until all tissue lysed, longer incubation time will increase yield of some FFPE tissue.) Brief spin down.
7. Incubate at 80°C for 15 min.
8. Add 225 µl Buffer RFB in to the lower phase, mix gently by pipetting.
9. Centrifuge at 11,000 x g for 1 min.
10. Aspirate 400 µl lower clear phase lysate into a new 1.5 ml micro-centrifuge tube.
11. Add 200 µl of 100% EtOH and mix thoroughly by vortex for 5 sec, brief spin down.
12. Transfer all mixture to Spin Column (with 2ml Tube), Centrifuge at 11,000 x g for 1 min. Discard the flow-through and change a new Collection Tube.
13. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
14. (Optional) On column digest of DNA with DNase I (not provided).
15. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
16. Add 700 µl Buffer RW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
17. Change a new Collection Tube, centrifuge at 11,000 x g for 3 min.
18. Place the spin column into 1.5 ml Elution Tube, add 30-100 µl RNase-Free H₂O and incubate at 25°C (room temperature) for 3 min.
19. Centrifuge at 11,000 x g for 1 min for elution.

FOR RESEARCH USE ONLY