CatchGene® FFPE Tissue DNA/RNA Kit

Cat. No.	Rxn
MT21004	4
MT21050	50

Kit Content

	4rxn	50rxn	
MT21 Column	8	100	pcs
Collection Tubes (2 ml)	24	300	pcs
Buffer AE	0.5	1.5	ml
Proteinase K	1x4	11x4	mg
Buffer DWX	2	27	ml
Buffer TFTL	2	27	ml
Buffer RFB	0.96	12	ml
Buffer RW1 (concentrate)	3.36	42	ml
Buffer RW2 (concentrate)	0.68	8.4	ml
RNase-Free H_2O	1	10	ml
Buffer DFL	0.96	12	ml
Buffer W1 (concentrate)	3.36	42	ml
Buffer W2 (concentrate)	0.68	8.4	ml
Elution Buffer	1	10	ml

Kit Storage

Upon arrival,

1. Please store **MT21 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 TFTL RFB or DFL, 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 (concentrate) 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 (concentrate) to get Buffer RW2.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.
5. **Prepare Buffer W1**
   Add equal volume of 100% EtOH into Buffer W1 (concentrate) to get Buffer W1.
   After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.
6. **Prepare Buffer W2**
   Add 4 volume of 100% EtOH into Buffer W2 (concentrate) to get Buffer W2.
   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 450 µl Buffer TFTL (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 40 µl Proteinase K (20 mg/ml) to the lower clear phase. Mix gently by pipetting.
6. Incubate at 60℃ for 30 min. Mix by finger flicking for every 10 min.
7. Centrifuge at 11,000 x g for 1 min.
8. Transfer 200 µl lower clear phase to a new 1.5 ml micro-centrifuge tube, proceed to **RNA Protocol** in below.
9. Leave all lysate the original tube and proceed to **DNA Protocol** in below.

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RNA Protocol

1. Incubate the 200 µl lower clear phase at 80℃ for 15 min.
2. Add 200 µl Buffer RFB, mix gently by pipetting.
3. Add 200 µl of 100% EtOH (not provided) and mix thoroughly by vortex for 5 sec, brief spin down.
4. Transfer all mixture to MT21 Column (with 2ml Tube), Centrifuge at 11,000 x g for 1 min. Discard the flow-through and change a new Collection Tube.
5. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
6. (Optional) On column digest of DNA with DNase I (not provided).
7. Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
8. Add 700 µl Buffer RW2 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
9. Change a new Collection Tube, centrifuge at 11,000 x g for 3 min.
10. Place the spin column into 1.5 ml Elution Tube, add 30-100 µl RNase-Free H_2O and incubate at 25°C (room temperature) for 3 min.
11. Centrifuge at 11,000 x g for 1 min for elution.

DNA Protocol

1. Go further Incubation with original lysate at 60 ℃ for 1h (or until the tissue has completely lysed). Brief spin down.
2. Incubate at 90℃ for 1h.
3. Centrifuge at 11,000 x g for 1 min.
4. Transfer 200 µl lower clear phase lysate (avoid to aspirate any debris) into a new 1.5 ml micro-centrifuge tube.
5. Add 200 µl Buffer DFL and mix by vortex for 5 sec. Briefly spin down than add 200 µl of 100% EtOH (not provided) and mix thoroughly by vortex for 5 sec.
6. Transfer all mixture to MT21 Column (with 2ml Tube), Centrifuge at 11,000 x g for 1 min. Discard the flow-through and change a new Collection Tube.
7. Add 700 µl Buffer W1 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
8. Add 700 µl Buffer W1 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
9. Add 700 µl Buffer W2 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
10. Change a new Collection Tube, centrifuge at 11,000 x g for 3 min.
11. Place the column into a 1.5 ml micro-centrifuge tube , add 30-200 µl Elution Buffer and incubate at 25°C (room temperature) for 3 min. Centrifuge at 11,000 x g for 1 min for elution.

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