

Kit Description

The CatchGene™ FFPE Tissue DNA kit provides a safer, quicker and more efficient silica membrane-based DNA 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 DNA Kit offers a safer and optimized xylene-free method for DNA purification from FFPE tissue. Furthermore, Buffer DFTL and DFL is able to lyse FFPE tissue efficiently, and eventually minimize vortex steps to prevent fragmentation and preserve high integrity of long DNA.

Kit Content

	4rxn	50rxn	250rxn	
MD21 Column	4	50	250	pcs
Collection Tube (2 ml)	12	150	750	pcs
PK Solvent	0.5	1.5	10	ml
Proteinase K	1x2	11x2	11x10	mg
Buffer DWX	2	27	135	ml
Buffer DFTL	1.5	18	90	ml
Buffer DFL	1.2	15	75	ml
Buffer W1 (concentrated)	3.4	42	210	ml
Buffer W2 (concentrated)	0.7	9	42	ml
Elution Buffer	1	12	60	ml

Kit Storage

Upon arrival,

1. Please store **MD21 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 DFTL or DFL, dissolve by incubating at 60°C.

Kit Preparation

1. **Prepare 20 mg/ml Proteinase K**
For 1 mg Proteinase K, please add 50 µl PK Solvent into tube and vortex thoroughly for dissolving.
For 11 mg Proteinase K, please add 550 µl PK Solvent 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.
2. **Prepare Buffer W1**
Add equal volume of 100% EtOH into concentrated Buffer W1 to get Buffer W1.
After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.
3. **Prepare Buffer W2**
Add 4 volume of 100% EtOH into concentrated Buffer W2 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 8 sections) in a 1.5 ml micro-centrifuge tube (not provided). Add 450 µl DWX buffer, vortex vigorously for 30 sec. Spin down to collect sample in the bottom.
2. Incubate at 90°C for 20 min. After incubation, allow to cool at room temperature (15-25°C).
3. Add 300 µl DFTL Buffer and mix thoroughly by vortex 5 sec.
4. Centrifuge at 11,000 x g for 1 min. (After centrifugation, sample will separate into two layers. Upper layer is in yellow color which mainly Buffer DWX. Lower layer is colorless which mainly Buffer DFTL and tissue debris.)
5. Add 20 µl PK to the lower clear phase. Mix gently by pipetting.
6. Incubate at 56°C for 1h. (or until the tissue has completely lysed).
7. Incubate at 90°C for 1h. (Please avoid leaving samples in the incubator and heating it from 56°C to 90°C. Please place take samples out from incubator and place them back while the incubator reach 90°C. Otherwise it will affect the result of de-cross-linking.)
8. Centrifuge at 11,000 x g for 1 min.
9. Transfer 250 µl lower clear phase lysate (avoid to aspirate any debris) into a new 1.5 ml micro-centrifuge tube.
10. Add 250 µl Buffer DFL and mix by vortex for 5 sec. Briefly spin down than add 250 µl of 100% EtOH and mix thoroughly by vortex for 5 sec.
11. Place the MD21 Column into a new Collection Tube (2 ml).
12. Transfer all lysate to MD21 Column, centrifuge at 11,000 x g for 1 min, discard the flow-through and change a new collection tube.
13. Add 700 µl Buffer W1 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
14. Add 700 µl Buffer W1 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
15. Add 700 µl Buffer W2 into the Column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
16. Change a new Collection Tube, centrifuge at 11,000 x g for 3 min.
17. 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.

FOR RESEARCH USE ONLY