INSTRUCTIONS

Magnetic FFPE Tissue RNA Extraction Kit

Catalog Number：RE06001　RE06002　RE0696B

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【Introduction】

EmerTher Magnetic FFPE RNA Extraction Kit is used for extracting RNA from formaldehyde fixed-paraffin embedded (FFPE) tissue.

The Kit contains superparamagnetic nanoparticles which are efficiently bound to nucleic acids and a safe and eco-friendly extraction system. The magnetic beads are coated through a unique process, enabling strong binding with nucleic acids and easy elution. The experimental procedure is simple and efficient: 1) following pretreatment, add sample to a lysis-binding buffer to enable cell lysis, RNA release and RNA binding to magnetic beads in one step; 2) apply magnetic force enabling easy wash of the beads with buffers; 3) elution of RNA from the beads using water or TE buffer. The extraction procedure is fully compatible with automation.

Purified RNA can be directly used in a variety of downstream experiments, including PCR, gene sequencing, etc.

【Product Features】

- Eliminate the need for xylene, a toxic solvent commonly used for dissolving paraffin
- Eliminate the use of toxic chloroform and phenol
- Convenient: No tissue grinding is necessary
- Effectively remove cross-linking between nucleic acids and proteins formed during formalin fixation, preventing inhibition of reverse transcription of the extracted RNA due to cross-linkage and increasing the sensitivity and consistency of subsequent tests
- Retain large RNA pieces, facilitating subsequenttesting
- Can be used with EmerTher magnetic FFPE tissue DNA extraction kit to extract DNA and RNA from one sample
- Automation: compatible with a variety of automatic magnetic bead processors; pre-packed plates are available

【Components】

Catalog No.	***RE06001 (20 preps)***	***RE06002 (100 preps)***	***RE0696B (96 preps)***
Format	bottles	bottles	pre-packed plates
Paraffin Sample Solution	10 ml	40 ml	40 ml
Paraffin Digestion Solution	3 ml	15 ml	15 ml
ATL Buffer	3 ml	15 ml	15 ml
PK Dissolving Solution	200 µl	1 ml	1 ml
Proteinase K	4 mg	20 mg	20 mg
Magnetic Bead Suspension	0.6 ml	3 ml	6 pre-packed plates, 12 tip combs (8-channels each)
Lysis-binding Buffer	12 ml	60 ml	
Wash Solution Ⅰ	12 ml	60 ml	
Wash Solution Ⅱ	12 ml	60 ml	
Elution Solution	1.5 ml * 2 bottles	1.5 ml * 8 bottles	

【Storage and Expiration】

1. The kit is shipped at room temperature. Upon receipt, please store proteinase K, PK Dissolving Solution and Elution Solution at -20°C;
2. Prior to use, prepare a proteinase K solution by adding PK Dissolving Solution to the bottle containing proteinase K, mix well and store the solution at -20°C;

3. The remaining reagents can be stored at room temperature for 12 months.

【Companion Device】

A water bath; a centrifuge; a magnetic separation rack and a rotary mixer (for manual extraction), or an automatic magnetic particle processor/liquid handler (for automatic extraction).

【Experiment Procedure】

A. Paraffin Dissolving:

1. Mix proteinase K and PK Dissolving Solution to prepare a proteinase K solution (20 mg/ml);
2. Transfer FFPE tissue (e.g. 1-5 pieces of 10 μm-thick FFPE tissue samples) into a 1.5 ml centrifuge tube;
3. Add 300 μl Paraffin Sample Solution, 150 μl ATL Buffer and 10 μl proteinase K solution, mix well and incubate for 1 hour at 56°C in a water bath. Shake the tube 3-5 times during the incubation. Overnight incubation is an alternative option;

B. Digestion:

4. Add 150 μl Paraffin Digestion Solution to mix with the sample, and incubate for 1 hour at 85°C in a water bath;

C. RNA Extraction:

5. Centrifuge at 15,000 g for 5 min;
6. Pipette the liquid at the bottom layer carefully to another 1.5 ml centrifuge tube. Add 600 μl Lysis-binding Buffer and 30 μl Magnetic Bead Suspension to the sample. Place the tube on a rotary mixer and mix for 15 min;
7. Place the tube on a magnetic separation rack, so beads are pulled to the side of the tube. Discard supernatant.
8. Add 600 μl Wash Solution I, gently invert the tube for 1 min to wash the beads. Place the tube back to the magnetic separation rack, discard supernatant.
9. Add 600 μl Wash Solution I again to wash the beads for 1 min. Place the tube back to the magnetic separation rack, discard supernatant.
10. Add 600 μl Wash Solution II to wash the beads for 1 min. Place the tube back to the magnetic separation rack, discard supernatant.
11. Add 600 μl Wash Solution II again to wash the beads for 1 min. Transfer the solution with beads into a new centrifuge tube. Place the tube back to the magnetic separation rack, discard supernatant.
12. Let the beads stand for 3-5 min to remove residual ethanol.
13. Add 100 μl Elution Solution and mix with beads for 5 min to release RNA from the beads. The volume of the elution solution can be reduced if a high concentration of RNA is desired.
14. Place the tube back to the magnetic separation rack and transfer supernatant containing RNA to a clean RNase-free centrifuge tube for subsequent testing or storage at -20°C. For long term storage, please store RNA at -70°C or add RNase inhibitor.

[Steps 6-14 can be completed on an automatic magnetic bead processor/liquid handler.]

【Additional Information】

1. The Lysis-binding Buffer and the Paraffin Digestion Solution may precipitate after long-term storage at low temperature. If the temperature is below 15°C, please warm the solutions at 37°C in a water bath until they turn to clear solutions before use.
2. The Lysis-binding Buffer and the Paraffin Digestion Solution contain protein denaturing agents, thus they are corrosive. Please handle them carefully. If such a solution is accidentally splashed on skin, please wash with plenty of water.
3. RNA is readily biodegradable, please use RNase-free tubes and pipette tips. Purified RNA products should be used for testing right after the extraction is complete, or stored at -20°C. For long term storage, please store RNA at -70°C or add RNase inhibitor.
4. Gently invert the tube during wash to ensure sufficient mixing. This helps increase the purity of extracted RNA.
5. Remove Wash Solution II completely before adding Elution Solution. Let beads stand for 3-5 min to remove residual ethanol. Too much ethanol residual may affect subsequent tests. But if the sample is too dry, it may be difficult for RNA to be eluted from beads and the yield may be reduced.
6. Elution step can be performed at room temperature.
7. The volume of the elution solution can be reduced to increase RNA concentration in the eluent, but may decrease the total extraction amount. In general, using the same total elution volume with repeated elution processes (a smaller elution volume is used at each time) can increase the elution efficiency and the total extraction amount.
8. Gently shake the centrifuge tube during elution step can help nucleic acid dissolve in solution.
9. Avoid freezing, drying and centrifugation at a high speed during use and storage of beads. Otherwise, this may decrease the binding capacity of the beads.

Automation procedure and setup for RE0696B

1. Setup for pre-packed 96 deep-well plates (extraction of 16 samples per run)

Columns 1,7	Columns 2,8	Columns 3,9	Columns 4,10	Columns 5,11	Columns 6,12
600 µl Lysis-binding Buffer Sample (to be added by user)	600 µl Wash Solution I; 30 µl magnetic beads	600 µl Wash Solution I	600 µl Wash Solution II	600 µl Wash Solution II	100 µl Elution Solution

2. After centrifugation, transfer the liquid at the bottom layer to columns 1 and 7, which are prefilled with the Lysis-binding Buffer (If room temperature is below 15°C, please warm the plate at 37°C for 20 min to ensure complete dissolution of any precipitates before use.).
3. Install two sets of tip combs (8 tip combs per set), select the automation procedure listed in the below table and start to extract nucleic acids.

Sequence	Column No.	Function	Mixing time (sec.)	Magnetic adsorption time (sec.)	Waiting time (sec.)	Volume (µl)	Mixing speed	Temp (℃)
1	1	Lysis	300	0	0	800	fast	37
2	2	Transfer Beads	20	20	0	600	fast	Room Temp
3	1	Binding	120	20	0	800	slow	37
4	2	Wash 1	120	20	0	600	fast	Room Temp
5	3	Wash 2	120	20	0	600	fast	Room Temp
6	4	Wash 3	60	20	0	600	fast	Room Temp
7	5	Wash 4	60	30	120	600	fast	Room Temp
8	6	Elution	300	60	0	100	fast	50
9	4	Move beads	30	0	0	600	fast	Room Temp

4. The elution solutions containing purified nucleic acids are transferred out from the columns 6 and 12, and used for subsequent testing or storage at -20°C. For long term storage, please store RNA at -70°C or add RNase inhibitor.

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