Ver. 25091

ODP439

FFPE RNA Kit

For RNA extraction from formalin fixed, paraffin embedded tissues

Origin

ISO 13485:2016 ISO 9001:2015

FFPE RNA Kit

ODP439

Kit Contents

Contents	50Preps
Buffer RF	8mL
Proteinase K (20mg/mL)	500µL
Buffer LB1	10mL
Buffer PW	15mL
Buffer GW	13mL
RNase-free Water	10mL
Acryl Carrier	2 × 500µL
RNase-free Spin column CR3 with Collection tubes (2mL)	50

Storage

FFPE RNA Kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Proteinase K should be stored at -20°C

Acryl Carrier should be stored at 2 -8°C

Introduction

FFPE RNA Kit is optimized for purification of RNA from formalin fixed and paraffin embedded tissue sections. It uses xylene to remove paraffin, and provides unique lysis conditions for RNA release from tissue slice, well removes formalin crosslinking of the released RNA. Combined selective-binding silica-based membrane and flexible elution system, this kit could elute high-quality RNA.

RNA purified by FFPE RNA Kit is stable and of high purity and is suited for PCR and Real-time PCR analysis, SNP genotype analysis and STR analysis, and pharmacogenomics analysis.

Important Notes

1. Fix tissue samples in 4-10% formalin for 8-24 hours immediately after surgical removal. Longer fixation time will lead to severe RNA fragmentation, resulting in poor performance in downstream assays.
2. Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit PCR reaction).
3. The integrity of RNA obtained with this kit depends on the samples type, storage and fixation conditions. Longer fixation time or longer storage time (>1 year) will lead to RNA fragmentation and in this case, long fragments will not be amplified.
4. Add appropriate amount of ethanol (96-100%) to Buffer GW and Buffer PW as indicated on the bottle before use.
5. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
6. Increasing the time of absorption and elution could improve recovery efficiency.
7. The recovery efficiency is related to starting RNA quantity and elution volume.
8. If a precipitate has formed in Buffer GW, warm buffer to 56°C until the precipitate has fully dissolved.

Protocol

Ensure that Buffer GW and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. **Preparation of samples**
   a) Paraffin section: 5-8 pieces of paraffin sections (thickness of 5-10μm, surface area of 1×1 cm^2).
   b) Paraffin block: Use a scalpel to cut around 30mg tissue sample (trim excess paraffin off).
   Note: If sample surface has been exposed to air, discard the first 2-3 sections.

- Place the paraffin section or paraffin block in a 1.5mL centrifuge tube, and add 1mL xylene to the sample. Close the lid and vortex vigorously for 10 seconds.
- Centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes at room temperature. Remove the supernatant by pipetting.
- **Note: Do not remove any pellet.**
- Add 1ml ethanol (96-100%) to the pellet and vortex for 10 seconds.
- Centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes at room temperature. Remove the supernatant by pipetting.
- **Note: Do not remove any pellet.**
- Keep the tube opened at room temperature for 5-10 minutes or until all the residual ethanol has evaporated.

c) Formalin fixed tissue: Use a scalpel to cut around 30mg tissue sample into small pieces and place in a 1.5mL microcentrifuge tube. Add 500µL PBS (10mM; pH 7.4) mix by vortexing and centrifuge for 1 minute at 12,000 rpm (~13,400 ×g), discard the supernatant and wash the pellet with 500µL PBS.

- Add 200µL NaCl (3M; pH 7.0) mix by vortexing and centrifuge for 1 minute at 12,000 rpm (~13,400 ×g), discard the supernatant and wash the pellet with 200µL NaCl.

2. Re-suspend the pellet in 150µL Buffer RF.
3. Add 10µL Proteinase K, mix thoroughly by vortexing.
4. Incubate the sample at 56°C for 15 minutes or until the tissue is completely lysed, followed by 80°C for 15 minutes to remove the cross-linked Proteinase K.
5. Incubate the samples in ice for 3 minutes and centrifuge at 10,000 rpm for 10 minutes.
6. Transfer the supernatant to a new 1.5mL microcentrifuge tube.
   Optional step: Treat the samples with DNase I (RNase-free) (Cat# ORT411) to remove the DNA.
7. Add one volume of Buffer LB1 to the supernatant and mix thoroughly by vortexing.
8. Add 20µL of Acryl Carrier and mix the contents by vortexing and incubate at room temperature for 10 minutes.
9. Add 2 volumes of Ethanol (96-100%) to the sample, close the cap and mix thoroughly by vortexing for 15 seconds.
   Note: Cool ethanol (96-100%) on ice before use if the room temperature is more than 25°C.
10. Pipette the mixture into the spin column (in a 2mL collection tube), keep the spin column CR3 for 5 minutes at room temperature and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through and place the spin column CR3 back to the collection tube.
11. Add 500µL Buffer GW to spin column CR3 and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through and place the spin column back to the collection tube.
12. Add 700µL Buffer PW to spin column CR3 and centrifuge 10,000 rpm for 1 minute. Discard the flow-through and place the spin column back to the collection tube.
13. Re-centrifuge the spin column CR3 placed in collection tube at 10,000 rpm for 2 minutes and discard the flow-through.
14. Place the spin column CR3 to a new 1.5mL micro-centrifuge tube and pipette 50µL RNase-free Water directly to the center of membrane. Incubate at room temperature (15-25ºC) for 3 minutes. Then centrifuge at 10,000 rpm for 1 minute.
15. The elute can be used for further downstream processes. For longer shelf life, store at -20°C.