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Prism FFPE RNA Extraction Kit

RNA Isolation Kit for FFPE Tissue Samples

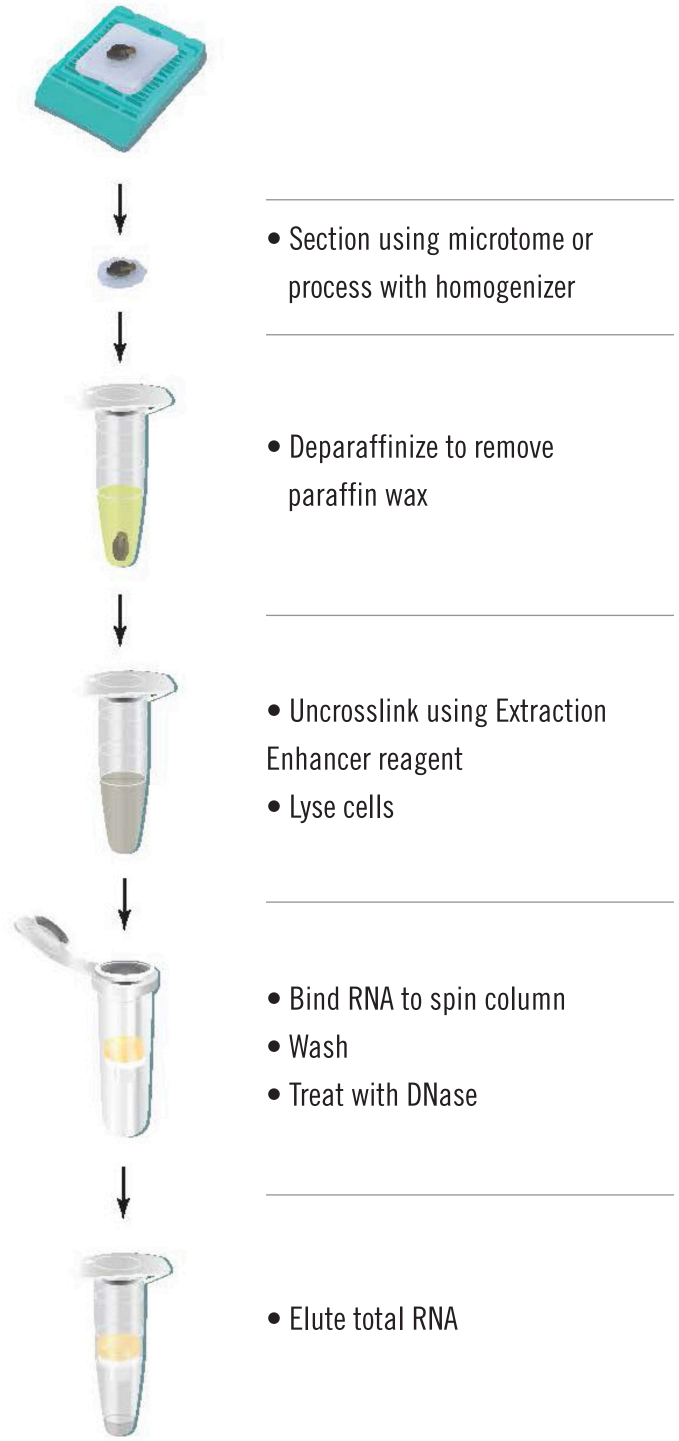
Kit Manual
24 reactions
Catalog #CFGK-401

Prism FFPE RNA Extraction Kit for Isolation of RNA from FFPE Tissue Samples

Introduction

Cofactor Genomics Prism FFPE RNA Extraction Kit provides a complete solution for obtaining RNA from challenging FFPE samples. The RNA extracted using this kit is suitable for use with the ImmunoPrism Immune Profiling Kit (Catalog #CFGK-301 and #CFGK-302).

This extraction method uses novel chemistry that improves the removal of formaldehyde-induced damage to the RNA molecules. As a result, larger molecules are obtained and accessible for use in downstream next generation sequencing applications.



Kit Contents

Reagent	Volume (24 reaction kit)
Extraction Enhancer	2.5 mL
Lysis Buffer	2.5 mL
Deparaffinization Reagent	15 mL
Binding Buffer	8 mL
DNase Buffer	2 mL
Wash Buffer	6 mL – Prior to use add 24 mL Ethanol
Protease	300 µL
DNase (lyophilized)	Prior to use, reconstitute in 60 µL Nuclease Free Water
Spin Columns	24 ea

Storage Conditions

Store the protease at 2-8 °C for prolonged shelf life. Reconstituted DNase I solution should be stored at -20 °C.
All other reagents should be stored at room temperature.

⚠ Safety

Use of this product in a manner inconsistent or not specified in the provided instructions may result in personal injury or damage to equipment. Prior to use, ensure that all users have received training and are familiar with general laboratory safety procedures as well as the specific safety information associated with this product. Safety data sheets can be accessed at *https://cofactorgenomics.com/product/immunoprism-kit/*.

Support

For troubleshooting, support, or questions, visit *https://support.cofactorgenomics.com/*
or e-mail *support@cofactorgenomics.com*.

Before You Start

Ensure that you have the following supplies and equipment that are not included with this kit:

- Microtome for tissue sectioning
- If not using the included Deparaffinization Reagent, a suitable alternative should be available (e.g. xylenes)
- Ethanol (200 proof, molecular biology grade)
- Heat block set to 72 □C
- Cold block or bucket of ice
- Low binding 1.5 mL microcentrifuge tubes (e.g. Eppendorf DNA/RNA LoBind recommended)
- Microcentrifuge (12,000 rcf minimum)
- Nuclease-free water for DNase I reconstitution and RNA elution step
- Wash Buffer: ensure that 24 mL of 200 proof ethanol has been added to the 6 mL of Wash buffer provided
- DNase I: Reconstitute the lyophilized DNase I by adding 60 µL of nuclease-free water. Gently pipette the solution up and down to fully reconstitute. Briefly spin down the tube to collect the contents at the bottom of the tube and incubate for 5 minutes at room temperature before returning to ice. To avoid repeated freeze/thaw events, it is helpful to generate aliquots. Store the aliquots at -20 °C.

Preparing the Tissue

FFPE tissue sections can be between 5-10 µm thick. For each isolation, 1 to 4 sections should be used. The tissue surface area within the FFPE block should be between 10 and 100 mm^2. The Cofactor ImmunoPrism Immune Profiling Kit requires between 20 - 40 ng of RNA, depending on quality.

RNA Extraction Protocol

Option A: Deparaffinization using Included Deparaffinization Reagent (Recommended)

The recommended option relies on the included Deparaffinization reagent, which is efficient and non-toxic. A fume hood is not required to perform this step. An alternative deparaffinization protocol using xylenes is included below in Option B.

A1. Place 1-4 FFPE sections in a 1.5 mL Eppendorf tube.

A2. Add 500 µL of Deparaffinization Reagent.

A3. Invert the tube several times to mix, then centrifuge briefly to bring sample to the bottom of the tube.

A4. Incubate samples at 72°C for 3 minutes, then allow to cool to room temperature.

A5. Add 80 µL of Extraction Enhancer to each tube containing the deparaffinized tissue sections.

A6. Invert tube several times to mix, then centrifuge for 1 minute at 16,000 rcf. At the bottom of the tube, a clear aqueous phase should form containing the tissue. The bright yellow upper organic phase contains the Deparaffinization Reagent and dissolved paraffin. If the tissue appears to be present in the upper organic phase, invert the tube (or vortex briefly) and centrifuge again until all the tissue is contained in the lower aqueous phase.

A7. Using a P1000 pipette, carefully remove most of the upper organic phase (approximately 100 µL can remain). Discard the aspirated upper organic phase.

A8. Proceed to Lysis and Crosslink Reversal (Step 1).

Option B: Deparaffinization using Xylenes

⚠ **Xylene fumes are toxic and should not be inhaled. Perform the following steps in a fume hood.**

B1. Place 1-4 FFPE sections in a 1.5 mL Eppendorf tube.

B2. In a fume hood, add 1 mL xylenes and close tube lid. Invert the tube several times to mix, then centrifuge at 16,000 rcf for 5 minutes.

B3. Remove the xylenes, taking care not to disturb the pellet.

B4. Add 1 mL of 200 proof ethanol, briefly vortex the sample, and centrifuge for 2 minutes at 16,000 rcf.

B5. Remove and discard the ethanol supernatant, taking care not to disturb the pellet.

B6. Repeat Steps B4 and B5 for a total of two ethanol washes.

B7. Let the tubes stand for 10 minutes to allow the residual ethanol to evaporate completely. Alternatively, a vacuum centrifuge (e.g., Speedvac®) can be used for quickly drying the samples.

B8. Add 80 µL of Extraction Enhancer to each tube containing the deparaffinized tissue sections and invert the tubes several times to mix. Perform a brief spin to ensure all the tissue is immersed in Extraction Enhancer solution.

B9. Proceed to Lysis and Crosslink Reversal (Step 1).

Lysis and Crosslink Reversal

1. Incubate tubes in a heat block at 72 °C for 30 minutes, then place on ice for 1 min.
2. To each tube, add 80 µL of Lysis Buffer, then add 10 µL of Protease directly to the aqueous phase. Gently invert the tube several times, then spin down briefly.
3. Incubate in a heat block at 72 °C for 2 hrs.
4. Place tubes on ice for 3 minutes.
5. Centrifuge tubes for 15 minutes at 16,000 rcf. A pellet will form containing cellular debris, while the RNA will remain in solution. The pellet may not be visible, but this will not affect the yield and quality of the obtained RNA.
6. Using a pipette, carefully transfer as much of the supernatant as possible to a new tube without disturbing the pellet. Bypass the organic layer by inserting the pipette tip along the wall of the tube.

RNA Binding

7. To each tube, add 150 µL of Binding Buffer, and then add 450 µL of ethanol. Mix well by inverting the tube several times.
8. Immediately transfer the content of each tube to a spin column (provided).
9. Centrifuge for 1 minute at 16,000 rcf. Discard flow-through.

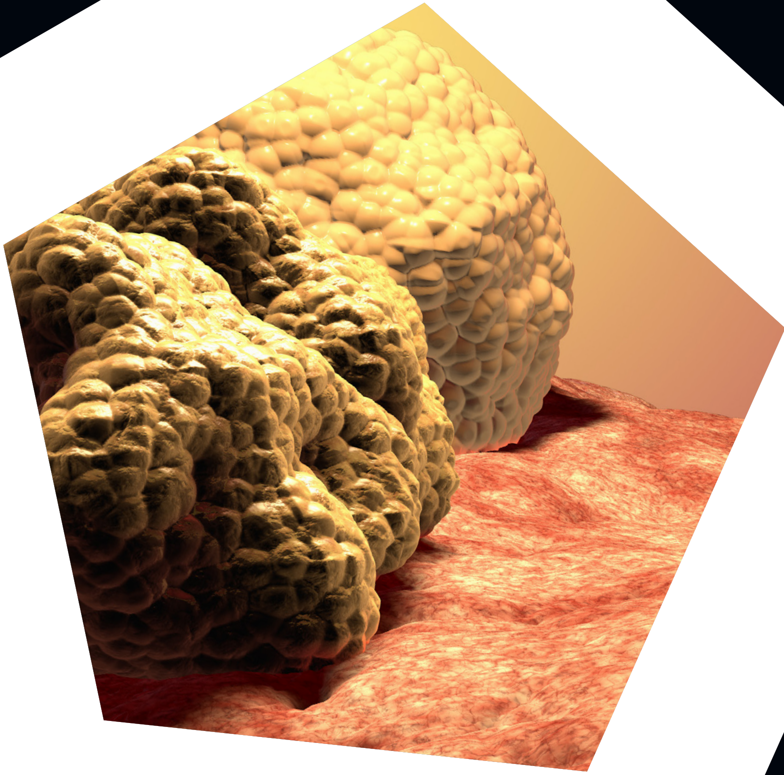
DNase I Treatment

This step removes any contaminating genomic DNA which may interfer with downstream applications.

10. Mix 120 µL of nuclease-free water, 120 µL of Binding Buffer and 360 µL of ethanol in a separate tube, for a total volume of 600 µL.
11. Add 300 µL of this mixture to the column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.
12. Mix 70 µL DNase I Buffer with 2 µL of reconstituted DNase I and add this mixture directly to the center of the membrane of the spin column. Let stand for 15 minutes at room temperature.
13. Add the remaining 300 µL of the Binding Buffer/ethanol mixture (prepared in Step 10) to the column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.

RNA Isolation

14. Add 500 µL of Wash Buffer to each spin column and centrifuge for 30 seconds at 16,000 rcf. Discard flow-through.
15. Repeat Step 14 for a total of 2 washes.
16. Dry the spin column by placing it back into an emptied collection tube and spinning again for 5 minutes at 16,000 rcf. Discard flow-through.
17. Place the column in a clean 1.5 mL microcentrifuge tube.
18. Elute the pure RNA by adding 50 µL of RNase-free water to the center of the membrane of the spin column. Let stand for 1 minute. Centrifuge for 1 minute at 16,000 rcf.
19. Eluted RNA should be stored at -80 °C.



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