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RNAstorm™

RNA Isolation Kit for FFPE Tissue Samples

Sample Kit (20 extractions)

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RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples

About the Kit

Biopsies and surgical specimens are routinely preserved by fixation with formaldehyde, in formalin-fixed paraffin-embedded (FFPE) tissue block format. While formaldehyde stabilizes tissue for storage, it also forms extensive crosslinks and adducts with nucleic acids in the sample. Such modifications strongly interfere with molecular analysis methods and must be removed.

Existing methods rely primarily on heat to remove crosslinks and adducts, which is only partially effective and leads to additional fragmentation of labile nucleic acids such as RNA. In contrast, the catalytic technology developed by Cell Data Sciences and included in the RNAstorm™ kit greatly accelerates the removal of formaldehyde damage and allows the use of milder conditions, resulting in markedly improved recovery of amplifiable RNA. This greatly enhances the chances of success in recovering high yields of good quality nucleic acids suitable for a variety of applications, including next-gen sequencing, qPCR, microarray, or other gene expression analysis.

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 of this product have received training in and are familiar with both general laboratory safety practices as well as the specific safety information associated with this product.

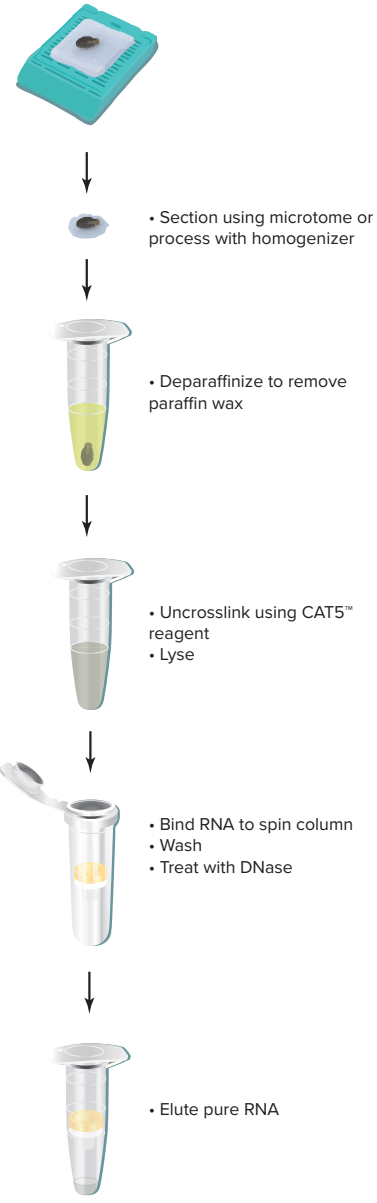
Safety information, including safety data sheets, can be accessed at http://celldatasci.com/safety.

Overview

Protocol Outline

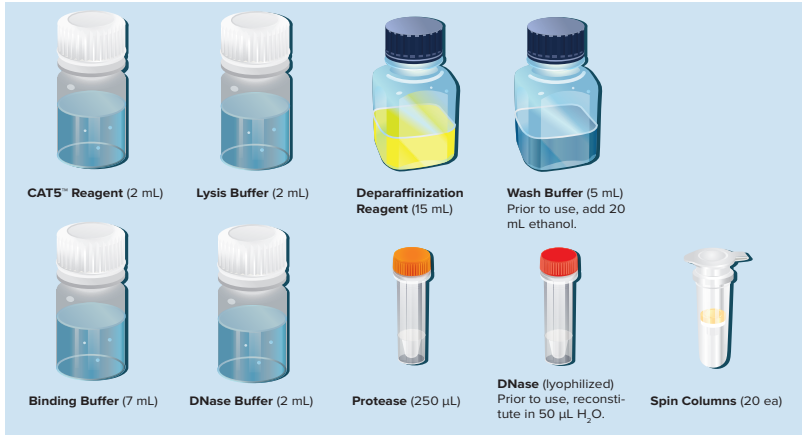
A typical RNA isolation involves the following steps:

- Preparation of Sections: very thin sections are cut from a block using a microtome, or the tissue is processed using a grinder or homogenizer.
- Deparaffinization: the paraffin is removed from the sections, leaving only the tissue.
- Uncrosslinking and Lysis: the tissue is treated to release RNA from other cellular components and to remove formaldehyde-induced modifications.
- DNase I Treatment: contaminating genomic DNA is degraded using DNase I. This step is optional but highly recommended.
- RNA Isolation: cellular debris and other impurities are removed from the RNA. The RNA is first bound to a spin column in the presence of Binding Buffer, then washed using Wash Buffer. Pure RNA is finally eluted using water.



Kit Contents

The following reagents are included with the RNAstorm kit:



Storage Conditions:

- All components of the kit should be stored at room temperature.
- For longer term storage, it is recommended to store protease solution at 2-8˚C for longer shelf life.
- After reconstitution, DNase I solution should be stored at -20˚C.

Before You Start

Please ensure that you have the following supplies and equipment ready, which are not provided with the kit:

- A microtome for tissue sectioning.
- If not using the included Deparaffinization Reagent, an alternative deparaffinization solution should be prepared or obtained in advance (e.g. xylenes).
- Ethanol (200 proof, molecular biology grade).
- Heat block set to 72˚C.
- An ice-filled container.

- 1.5 mL microcentrifuge tubes (Eppendorf® DNA/RNA LoBind recommended).
- Microcentrifuge (12,000 rcf minimum).
- RNase-free water for DNase I reconstitution and final RNA elution step.
- Wash Buffer: ensure that 20 mL of 200 proof ethanol has been added to the provided bottle.
- DNase I: Reconstitute the lyophilized DNase I by adding 50 µL of RNase-free water. Using a pipette, mix gently to ensure the DNase is fully reconstituted. Briefly spin down tube if needed. To avoid repeated freezing and thawing of DNase, it is helpful to make aliquots as needed. Store the aliquots at -20°C.

Preparing the Tissue

The RNAstorm™ kit can be used with FFPE sections between 5-10 µm thick. For each isolation, 1 to 4 sections should be used. The tissue should have an approximate total surface area between 10 and 100 mm^2. Alternatively, an equivalent amount of tissue may be ground or crushed using a tissue grinder/homogenizer or a small mortar and pestle.

Detailed RNA Isolation Protocol

Option A: Deparaffinization using Included Deparaffinization Reagent (Recommended)

This recommended procedure relies on the included Deparaffinization Reagent, which is efficient and non-toxic. A fume hood is not necessary to perform this step. An alternative deparaffinization protocol using xylenes is included below (Option B).

A1. Place the desired number of sections in a 1.5 mL Eppendorf tube.

A2. Add 500 µL of Deparaffinization Reagent.

A3. Vortex for 10 seconds, 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 at room temperature.

A5. Add 80 µL of CAT5™ Reagent to each tube containing deparaffinized tissue sections.

A6. Vortex vigorously for 10 seconds, then centrifuge for 1 minute at 16,000 rcf. At the bottom of the tube, a clear aqueous phase should form containing the tissue, along with a bright yellow upper organic phase containing the Deparaffinization Reagent and paraffin. If tissue appears to be present in the upper organic phase, vortex and centrifuge again until all tissue is contained in the lower aqueous phase.

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

A8. Centrifuge tubes briefly, then proceed to step 1.

Option B: Deparaffinization using Xylenes

⚠ Xylene fumes are toxic and should not be inhaled. Perform these steps in a suitable fume hood.

B1. Place the desired number of sections in a 1.5 mL Eppendorf tube.

B2. In a fume hood, add 1 mL xylenes and close tube lid. Vortex for 10 seconds, then centrifuge at 16,000 rcf for 5 minutes.

B3. Remove the xylenes, being careful not to disturb the pellet.

B4. Add 1 mL ethanol, then vortex 10 seconds and centrifuge at 16,000 rcf for 2 minutes.

B5. Remove and discard the ethanol, being careful not to disturb the pellet.

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

B7. Let stand at room temperature for 10 minutes for the residual ethanol to evaporate completely. Alternatively, a SpeedVac may be used for quick drying of the samples.

B8. Add 80 µL of CAT5™ Reagent to each tube containing deparaffinized tissue sections and vortex for 10 seconds.

B9. Proceed to step 1.

Uncrosslinking and Lysis

1. Incubate 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. Vortex briefly, then spin down briefly.
3. Incubate in a heat block at 72˚C for 2 hours.
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. *Note if using Deparaffinization Option A*: following step A7, a thin organic layer may still be present, but will not interfere with the isolation procedure. Bypass this organic layer by inserting the pipette tip along the wall of the tube.

Begin RNA Isolation

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

DNase I Treatment (Recommended)

This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 14.

10. Mix 120 µL of RNase-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.

Continue 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. Wash again by repeating step 14.
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 Eppendorf 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.

Support

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Additional technical information as well as Frequently Asked Questions can be found at http://celldatasci.com/faq.

Literature

- Karmakar S et al., Organocatalytic Reversal of Formaldehyde Adducts of RNA and DNA Bases, *Nature Chemistry* **2015**, 7, 752-758.

Ordering Information

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