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Product Information

RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples

Catalog Numbers: CD201 (20 preps)
CD501 (50 preps)

Kit Components

Component	CD201 20 preps	CD501 50 preps
CAT5™ Reagent	2 mL	5 mL
Lysis Buffer	2 mL	5 mL
Deparaffinization Reagent	15 mL	30 mL
Wash Buffer (Prior to use, add ethanol)	5 mL (add 20 mL ethanol)	12 mL (add 48 mL ethanol)
Binding Buffer	7 mL	15 mL
DNase Buffer	2 mL	5 mL
Protease	250 uL	600 uL
DNase, lyophilized (Prior to use, reconstitute in H_2O)	(add 50 uL H_2O)	(add 120 uL H_2O)
Spin Columns	20 ea	50 ea

Storage Conditions

- 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.
- All other components of the kit should be stored at room temperature.

About the Kit

Biopsies and surgical specimens are routinely preserved by fixation with formadehyde, 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 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.

References

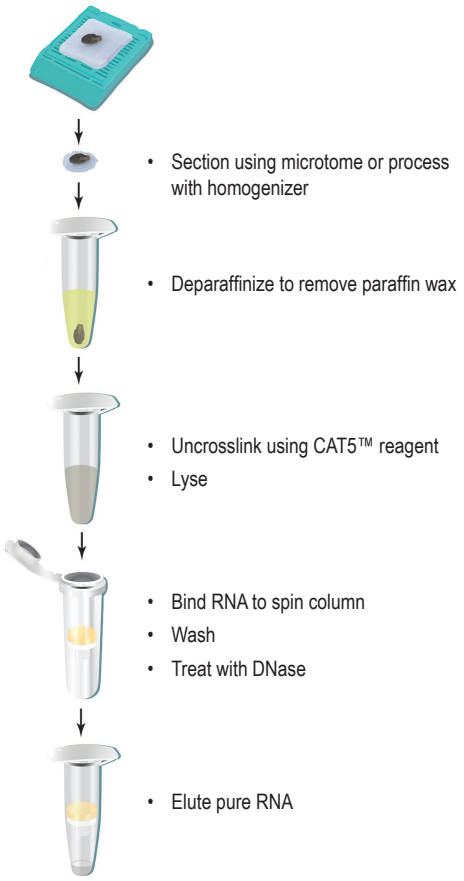
Karmakar S et al., Organocatalytic Reversal of Formaldehyde Adducts of RNA and DNA Bases, Nature Chemistry 2015, 7, 752-758.

Wehmas, LC et al., Demodifying RNA for Transcriptomic Analyses of Archival Formalin-Fixed Paraffin-Embedded Samples, Tox Sci 2017, 1-13. doi: 10.1093/toxsci/kfx278.

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.



- Section using microtome or process with homogenizer
- Deparaffinize to remove paraffin wax
- Uncrosslink using CAT5™ reagent
- Lyse
- Bind RNA to spin column
- Wash
- Treat with DNase
- Elute pure RNA

Materials required but not supplied

- 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.

Before you begin

Prepare the following buffers

- Wash Buffer: ensure that 20 mL (20 preps kit) or 48 mL (50 preps kit) of 200 proof ethanol has been added to the provided bottle.
- DNase I: Reconstitute the lyophilized DNase I by adding 50 uL (20 preps kit) or 120 uL (50 preps kit) 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.

Prepare the Tissue

The RNAstorm™ kit can be used with FFPE sections between 5-10 um 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)

Note: 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 microcentrifuge tube.

A2. Add 500 uL of Deparaffinization Reagent.

A3. Invert 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 at room temperature.

A5. Add 80 uL of CAT5™ Reagent to each tube containing 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, 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, invert tube (or vortex briefly) 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 uL can remain). Discard aspirated upper organic phase.

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

Option B: Deparaffinization using Xylenes

Note: 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 microcentrifuge tube.

B2. In a fume hood, add 1 mL xylenes and close tube lid. Invert tube several times to mix, 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 invert tube to mix. 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 centrifugal evaporator may be used for quick drying of the samples.

B8. Add 80 uL of CAT5™ Reagent to each tube containing deparaffinized tissue sections and invert tube several times to mix.

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 uL of Lysis Buffer, then add 10 uL of Protease. Invert tube gently several times to mix, 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 uL of Binding Buffer, and then add 450 uL 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)

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

10. Mix 120 uL of RNase-free water, 120 uL of Binding Buffer and 360 uL of ethanol in a separate tube, for a total volume of 600 uL.
11. Add 300 uL of this mixture to the column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.
12. Mix 70 uL DNase I Buffer with 2 uL 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 uL 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 uL 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 microcentrifuge tube.
18. Elute the pure RNA by adding 50 uL 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.

Related Products

Cat. #	Product
CD202	DNAstorm™ kit for isolation of DNA from FFPE tissue samples
CD504	RNAstorm™ RNA Isolation Kit
31030	DNA Gel Extraction kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
41042	DNAzure® Blue Nucleic Acid Gel Stain
E90003	Gel-Bright™ LED Gel Illuminator
31022	Ready-to-Use 1 kb DNA ladder
31032	Ready-to-Use 100 bp DNA ladder
31042	Forget-Me-Not™ qPCR master mix
31043	Forget-Me-Not™ Universal Probe Master Mix
31000	EvaGreen Dye®, 20X in water

Please visit our website at www.biotium.com for information on our life science research products, including qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.