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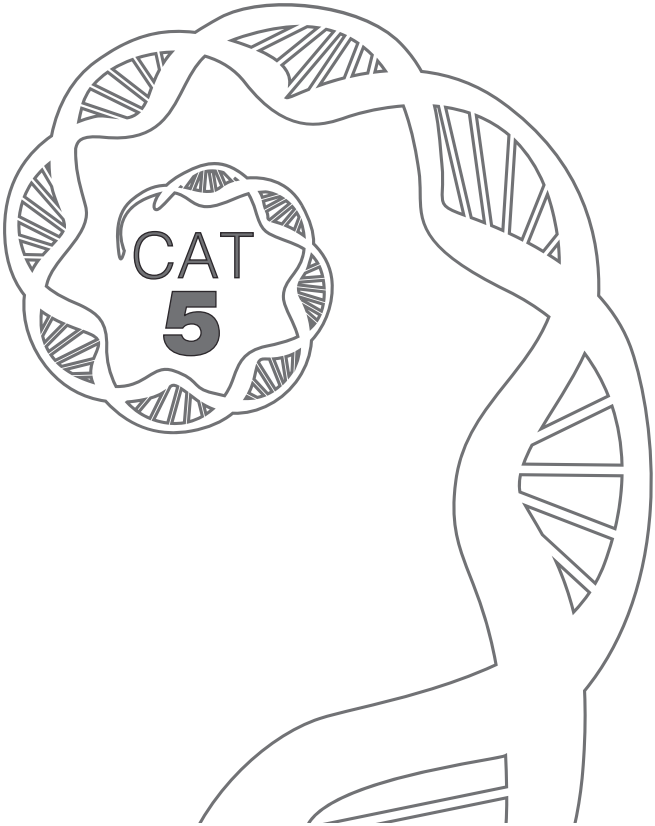
https://celldatasci.com
info@celldatasci.com

DNAstorm™ FFPE

DNA Isolation Kit for FFPE Tissue Samples

Kit Manual

20 extractions (CD202)
50 extractions (CD502)



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Support:

Email:	support@celldatasci.com
Phone:	650.285.2376 (option 2)
Toll-free:	855.620.7300 (option 2)
Web:	celldatasci.com/faq

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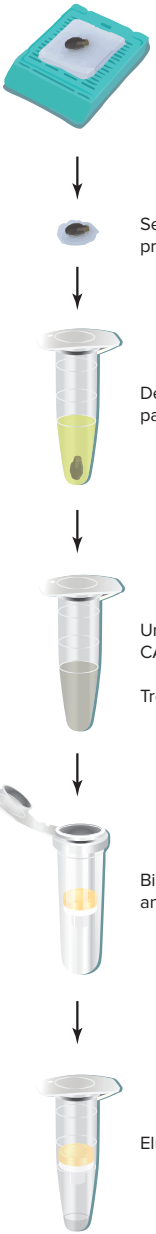
DNAstorm™ Kit for Isolation of DNA from FFPE Tissue Samples

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 and denaturation of double-stranded DNA. In contrast, the catalytic technology developed by Cell Data Sciences and included in the DNAstorm™ kit greatly accelerates the removal of formaldehyde damage and allows the use of milder conditions, resulting in markedly improved recovery of amplifiable DNA. 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 and qPCR.



⚠ 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 https://celldatasci.com/safety.

Overview

Protocol Outline

The DNAstorm™ isolation protocol 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 DNA from histone proteins and other cellular components and to remove formaldehyde-induced modifications.
- RNase A Treatment: contaminating RNA is degraded using RNase A. This step is optional but highly recommended.
- DNA Isolation: cellular debris, proteins, and other impurities are removed from the DNA. The DNA is first bound to a spin column in the presence of Binding Buffer, then washed using Wash Buffer. Pure DNA is finally eluted using water or a low-salt buffer.

Kit Contents

The following reagents are included with the DNAstorm kit:

Reagent	Volume (20 rxn kit)	Volume (50 rxn kit)
 CAT5™ Lysis Buffer	5 mL	12 mL
 Deparaffinization Reagent	15 mL	30 mL
 Binding Buffer	7 mL	15 mL
 Wash Buffer	5 mL. Prior to use, add 20 mL ethanol.	12 mL. Prior to use, add 48 mL ethanol.
 Proteinase K	500 µL	1.2 mL
 RNase A	250 µL	600 µL
 Spin Columns	20 ea	50 ea

Storage Conditions:

- All components of the kit should be stored at room temperature. For longer term storage, it is recommended to store Proteinase K and RNase A at 2-8˚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 in advance (e.g. xylenes).
- Ethanol (200 proof, molecular biology grade).
- Heat blocks set to 56˚C and 80˚C.
- An ice-filled container.
- 1.5 mL microcentrifuge tubes (Eppendorf® DNA/RNA LoBind recommended).
- Microcentrifuge (12,000 rcf minimum).
- Wash Buffer: ensure that 20 mL (for the 20 reaction kit) or 48 mL (for the 50 reaction kit) of 200 proof ethanol has been added to the provided bottle.
- Elution Step: we recommend using Tris or Tris-EDTA buffer at pH 8 (not provided). Alternatively, nuclease-free water can be used.

Preparing the Tissue

The DNAstorm™ 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 mm². Alternatively, an equivalent amount of tissue may be ground or crushed using a tissue grinder/homogenizer or a small mortar and pestle.

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

A4. Incubate samples at 80°C for 3 minutes, then allow to cool at room temperature.

A5. Add 200 µL of CAT5™ Lysis Buffer to each tube containing deparaffinized tissue sections.

A6. 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 any tissue remains in the upper organic phase, mix gently, then 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 150 µL can remain). Discard aspirated upper organic phase.

A8. 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 200 µL of CAT5™ Lysis Buffer to each tube containing deparaffinized tissue sections and invert tube several times to mix. Briefly spin down tube and ensure that all tissue is completely immersed in CAT5™ Reagent.

B9. Proceed to step 1.

Uncrosslinking and Lysis

1. To each tube, add 20 µL of Proteinase K. If any organic phase remains following step A7, make sure to add the Proteinase K directly to the aqueous phase. Mix each tube briefly using a pipette, then spin down at 16,000 rcf for 30 seconds.
2. Incubate tubes in a 56˚C heat block for 1 hour.
3. Move the tubes to a 80˚C heat block and incubate for 4 hours.
4. Place tubes on ice for 1 minute.
5. Spin down briefly.
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.

RNase Treatment (Recommended)

This step ensures that any contaminating RNA is degraded by incubating the sample with RNase A. To skip this step, proceed to step 9.

7. Add 10 µL of RNase A and invert tube gently to mix. Incubate at room temperature for 15 minutes.

DNA Isolation

8. To each tube, add 200 µL of Binding Buffer, and then add 600 µL ethanol. Mix well by inverting the tube several times.
9. Promptly transfer 700 µL of each tube to a spin column.
10. Centrifuge for 1 minute at 16,000 rcf. Discard flow-through.
11. Transfer the remaining content of each tube to the spin column and repeat centrifugation in step 10.
12. Add 500 µL of Wash Buffer to each spin column and centrifuge for 30 seconds at 16,000 rcf. Discard flow-through.
13. Wash again by repeating step 12.
14. 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.
15. Place the column in a clean 1.5 mL Eppendorf tube.
16. Elute the pure DNA by adding 50 µL of Tris or Tris-EDTA buffer (pH 8) or nuclease-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.
17. Eluted DNA should be stored at -20˚C.

Literature

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

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Cell Data Sciences, Inc.
46127 Landing Pkwy
Fremont, CA 94538
https://celldatasci.com
info@celldatasci.com