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Product Information

DNAstorm™ Kit for Isolation of DNA from FFPE Tissue Samples

Catalog Numbers: CD202 (20 extractions)
CD502 (50 extractions)

Kit Components:

Component	CD202 20 extractions	CD502 50 extractions
CAT5™ Reagent	3 mL	6 mL
Lysis Buffer	3 mL	6 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
Proteinase K	250 uL	600 uL
RNase A	250 uL	600 uL
Spin Columns	20 ea	50 ea

Storage Conditions:

- It is recommended to store Proteinase K and RNase A solutions at 2-8˚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 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.

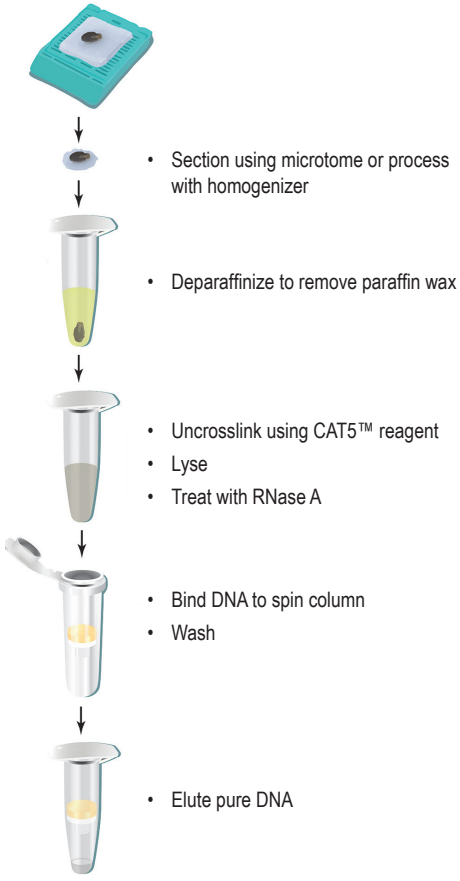
References:

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

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: RNA is degraded using RNase A. This step is optional but highly recommended.
- DNA Isolation: cellular debris 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.



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

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 blocks set to 56˚C and 72˚C.
- An ice-filled container.
- 1.5 mL microcentrifuge tubes (Eppendorf DNA/RNA LoBind recommended).
- Microcentrifuge (12,000 rcf minimum).
- Elution solution: we recommend using Tris or Tris-EDTA buffer at pH 8 (not provided). Alternatively, nuclease-free water can be used.

Before you begin

Prepare the following buffer

- Wash Buffer: ensure that 20 mL (20 extraction kit) or 48 mL (50 extraction kit) of 200 proof ethanol has been added to the provided bottle.

Prepare the Tissue

The DNAstorm™ 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 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 microcentrifuge tube.

A2. Add 500 uL 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 100 uL 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 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. 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 centrifugal evaporator may be used for quick drying of the samples.

B8. Add 100 uL 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 100 uL of Lysis Buffer, then add 10 uL of Proteinase K. Vortex briefly, then spin down at 16,000 rcf for 1 minute.
3. Incubate the tubes in a 56˚C heat block for 1 hour.
4. Move the tubes to a 72˚C heat block and incubate for 4 hours.
5. Place tubes on ice for 3 minutes.
6. Spin down briefly.
7. 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)

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

8. Add 10 uL of RNase A and incubate at room temperature for 15 minutes.

Begin DNA Isolation

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

Related Products:

Cat. #	Product
CD201	RNAstorm™ kit for isolation of RNA from FFPE tissue samples
31030	DNA Gel Extraction kit
31007	AccuBlue™ Broad Range dsDNA Quantitation Kit
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit
31060	AccuBlue™ NextGen dsDNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
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.