Revised: April 27, 2022

Product Information

CELLDATA DNAstorm™ FFPE DNA Extraction Kit

Catalog Number: CD502 (50 preps)

Kit Contents

Component	Size
99860-30ML: Deparaffinization Reagent	30 mL
99869-12ML: DNAstorm™ FFPE CAT5™ Lysis Buffer	12 mL
99870: DNAstorm™ FFPE Proteinase K	1.2 mL
99871-600UL: RNase A	600 uL
99863: CELLDATA FFPE Binding Buffer	15 mL
99864: CELLDATA Wash Buffer	12 mL (Add 48 mL ethanol prior to use)
99868-50: CELLDATA Spin Columns	50 each

Storage and Handling

Upon receipt, store Proteinase K and RNase A solutions at 2-8°C. Store other kit components at room temperature. Kit components are stable for at least 9 months from date of receipt when stored as recommended. The Binding Buffer contains the chaotropic salt guanidine hydrochloride, which is hazardous (see SDS). Use gloves and other appropriate laboratory protection when using this kit. Mixing bleach with guanidine hydrochloride can produce hazardous byproducts. DO NOT mix waste from this kit with bleach. Handle all kit components using universal laboratory safety precautions.

Product Description

Biopsies and surgical specimens are routinely preserved as formalin-fixed, paraffin embedded (FFPE) tissue blocks. While formaldehyde stabilizes tissue for storage and sectioning, 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 for efficient extraction of nucleic acids from FFPE tissue.

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 used in the CELLDATA DNAstorm™ FFPE DNA Extraction Kit greatly accelerates the removal of formaldehyde damage and allows the use of milder conditions, resulting in markedly improved recovery of amplifiable DNA. Compared to other methods, 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.

Evaluating FFPE DNA Extraction

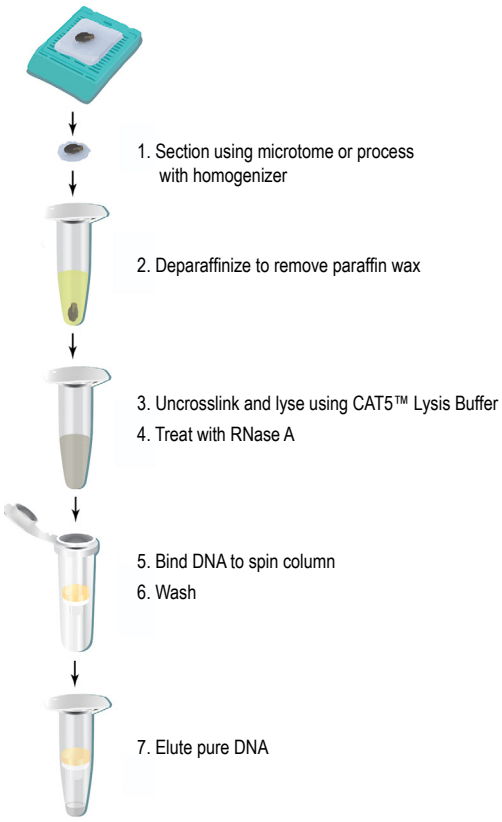
The following techniques may be used to evaluate the quality and quantity of your FFPE-derived DNA after extraction.

- **Concentration:** The DNA recovery will depend primarily on the amount and integrity of the tissue sample, but with samples of good quality it is possible to recover greater than 1 ug of total DNA per sample. Measurements can be obtained using UV/Vis spectroscopy (e.g., NanoDrop®) or using a DNA-specific fluorescence-based quantitation assay such as AccuBlue® NextGen dsDNA Quantitation Kit or AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit (see Related Products).
- **DNA Integrity:** Due to the wide size distribution of DNA isolated from FFPE tissue samples, we recommend using pulsed-field gel electrophoresis (PFGE) for evaluating DNA integrity. Methods based on capillary electrophoresis such as the Agilent Bioanalyzer can also be used, but may not properly resolve high molecular weight fragments (greater than 10 kb) in better-quality samples.
- **Amplifiability:** The standard method is quantitative real-time PCR expressed as a Ct number or as a relative or absolute amount of DNA. View our EvaGreen® Dye and Forget-Me-Not EvaGreen® qPCR Master Mixes (see Related Products). Please note PCR inhibition from residual chemical modifications and DNA damage is common when high amounts of FFPE-extracted template DNA are used. For tips to address this issue, see the FAQ section on page 3 “Why does my extracted DNA fail to amplify properly?”.

Protocol Outline

The DNAstorm™ FFPE DNA extraction procedure involves the following steps:

- **Preparation of sections**: Paraffin 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.
- **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.



Materials required but not supplied

- A microtome for tissue sectioning
- Optional: Xylenes may be used in place of the provided deparaffinization reagent (see Option B in protocol)
- 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 LoBind® tubes recommended)
- Microcentrifuge (12,000 x g minimum)
- Elution solution: We recommend using Tris or Tris-EDTA buffer at pH 8 (recommended) or nuclease-free water

Before you begin

Prepare the wash buffer

Add 48 mL of 200 proof ethanol to the bottle of CELLDATA Wash Buffer and mix well. Mark the ethanol added box on the label.

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. Tissue sections may be scraped off of slides using a razor blade and collected in a microcentrifuge tube for processing. 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 (recommended): Deparaffinization using included reagent

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 1 to 4 sections into a 1.5 mL microcentrifuge tube.

A2. Add 500 uL 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 the tube in a heat block at 80°C for 3 minutes, then cool to room temperature.

A5. Add 200 uL of DNAstorm™ FFPE CAT5™ Lysis Buffer to the tube containing deparaffinized tissue sections.

A6. Centrifuge for 1 minute at 16,000 x g. 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, mix gently by inverting the tube (do not vortex), and 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 uL can remain). Discard the upper organic phase.

A8. 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 1 to 4 sections in a 1.5 mL microcentrifuge tube.

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

B3. Remove all liquid, being careful not to disturb the pellet.

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

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

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

B7. Let the tube stand open 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 sample.

B8. Add 200 uL of DNAstorm™ FFPE CAT5™ Lysis Buffer to the tube containing deparaffinized tissue sections and invert the tube several times to mix gently (do not vortex). Briefly centrifuge the tube and ensure that all tissue is completely immersed in DNAstorm™ FFPE CAT5™ Lysis Buffer.

B9. Proceed to step 1.

1. Uncrosslinking and lysis

1.1 Add 20 uL of Proteinase K to the tube containing tissue. If any organic phase remains following step A7, make sure to add the Proteinase K directly to the aqueous phase. Mix the solution briefly by pipetting up and down, and then centrifuge at 16,000 x g for 30 seconds.

1.2 Incubate the tube in a 56°C heat block for 1 hour.

1.3 Move the tube to a 80°C heat block and incubate for 4 hours.

1.4 Place the tube on ice for 1 minute.

1.5 Centrifuge briefly to collect contents at the bottom of the tube.

1.6 Using a pipette, carefully transfer as much of the supernatant (containing DNA) as possible to a new tube, without disturbing the pellet. Discard the pellet.

Note: If using Deparaffinization Option A, 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.

2. 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 3.1.

2.1 Add 10 uL of RNase A to the supernatant from step 1.6 and incubate at room temperature for 15 minutes.

3. DNA isolation

3.1 To the tube, add 200 uL of CELLDATA FFPE Binding Buffer, and then add 600 uL of ethanol. Mix well by inverting the tube several times.

3.2 Promptly transfer 700 uL from the tube to a spin column.

3.3 Centrifuge for 1 minute at 16,000 x g. Discard the flow-through.

3.4 Transfer the remaining contents of the tube to the spin column and repeat centrifugation as in step 3.3.

3.5 Add 500 uL of CELLDATA Wash Buffer to the spin column and centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

3.6 Wash again by repeating step 3.5.

3.7 Dry the spin column by placing it back into an emptied collection tube and centrifuge again for 5 minutes at 16,000 x g. Discard the flow-through.

3.8 Place the column in a clean 1.5 mL microcentrifuge tube.

3.9 Elute the pure DNA by adding 50 uL of Tris or Tris-EDTA buffer (pH 8) or nuclease-free water to the center of the spin column membrane. Let stand for 1 minute. Centrifuge for 1 minute at 16,000 x g.

3.10 Optional: Repeat step 3.9 for a higher DNA yield but at a lower concentration.

3.11 Eluted DNA should be stored at -20°C.

Frequently Asked Questions (FAQs)

Question	Answer
Is there any contaminating RNA in the DNA obtained using the DNAstorm™ kit?	Contamination from RNA is eliminated by performing an optimized RNase digestion step immediately following the lysis step.
How much DNA can I expect to obtain from an FFPE sample?	The biggest variable that affects the total amount of DNA obtained is the quality of the sample itself (i.e. the type and amount of tissue, and the care taken in isolation and preservation of the sample). Using the DNAstorm™ kit, and assuming at least reasonable sample quality, amounts greater than 1 ug can be obtained.
Can DNA obtained using the DNAstorm™ kit be used in next-generation sequencing?	Yes. Good quality libraries can be obtained, providing that the DNA is of sufficiently high quality.
How should the tissue be prepared?	Use a microtome to obtain 5-10 um sections from FFPE samples. Sections thinner than 5 um may be used if they can be reliably cut. Sections thicker than 10 um are not recommended because they may not be fully digested.
Can I use tissue that is not paraffin-embedded?	Yes, tissue can be used which is not embedded in paraffin. In this case, we recommend mechanically grinding an amount of tissue equivalent to the recommended number of sections.
Can I use FFPE cores?	Yes, FFPE cores can be used. Because cores are not processed using a microtome, sample digestion tends to be more difficult and mechanical homogenization (e.g., using steel beads) is recommended if incomplete digestion is observed.
Which deparaffinization method do you recommend?	The DNAstorm™ kit includes a recommended Deparaffinization Reagent. Unlike other common methods (e.g., xylenes), the Deparaffinization Reagent is efficient, non-toxic and does not require the use of a fume hood. In our testing, the included reagent is at least as effective as xylenes at removing paraffin and allowing purification of high quality nucleic acids.
After mixing the Deparaffinization Reagent with the CAT5™ Lysis Buffer, I see a white cloudy layer in between the Deparaffinization Reagent layer and the aqueous layer. What is this and how does it affect the extraction?	The white cloudy layer is an emulsion between the Deparaffinization Reagent and the CAT5™ Lysis Buffer which may form when these two reagents are vortexed or given a hard mix. To avoid this issue, we recommend not vortexing the sample when the Deparaffinization Reagent and CAT5™ Lysis Buffer are in contact. When mixing is necessary in the presence of both these reagents (e.g., when protease is added), we recommend pipette mixing. The white cloudy layer can be removed by centrifuging at maximum speed (≥ 16,000 x g) for at least 2 minutes. The length of time will depend on the volume of the emulsion.
How can I evaluate the integrity of the DNA I obtained?	Due to the wide size distribution of DNA isolated from FFPE tissue samples, we recommend using pulsed-field gel electrophoresis (PFGE). Methods based on capillary electrophoresis such as the Agilent Bioanalyzer can also be used, but may not properly resolve high molecular weight fragments (greater than 10 kb) in better-quality samples.
Why does my extracted DNA fail to amplify properly? I notice a lot of PCR inhibition and/or Ct values that make no sense.	PCR inhibition is often observed when high amounts of FFPE-extracted template DNA are used. The inhibition is usually not due to the presence of contaminants, but results from residual chemical modifications and damage in the DNA itself. Several simple adjustments to the PCR protocol can overcome this issue. First, the amount of template DNA should be decreased. Second, the amount of PCR polymerase should be increased by 2-4X. Third, the annealing and extension times should be extended. Fourth, the amount of dNTPs can be increased. An in-depth discussion of this issue is found in Dietrich et al. (2013). PLoS ONE 8(10): e77771.

Related Products

Catalog number	Product
31030	DNA Gel Extraction Kit
CD501	CELLDATA RNAstorm™ FFPE RNA Extraction Kit
CD504	CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation 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
31022	Ready-to-Use 1 kb DNA ladder
31032	Ready-to-Use 100 bp DNA ladder
31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31043	Forget-Me-Not™ Universal Probe Master Mix
31073	AccuBlue® Broad Range RNA Quantitation Kit
31077	EvaGreen® Plus Dye, 20X in water
31000	EvaGreen® Dye, 20X in water
41024-4L	Water, Ultrapure Molecular Biology Grade

Please visit our website at www.biotium.com for information on our products for molecular biology workflows, including DNA/RNA extraction kits for fresh cells and FFPE tissues, nucleic acid quantitation kits, and nucleic acid gel stains.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.