Revised: October 11, 2023

# Product Information

## CELLDATA DNAstorm™ 2.0 MagBead FFPE DNA Extraction Kit, 96 preps

**Catalog Number:** CD509-96

### Kit Contents

| Component | Size |
|---|---|
| 99876-30ML: Dewaxing Solution | 2 x 30 mL |
| 99869-12ML: DNAstorm™ FFPE CAT5™ Lysis Buffer | 2 x 12 mL |
| 99870: DNAstorm™ FFPE Proteinase K | 2 x 1.2 mL |
| 99871-600UL: RNase A | 2 x 600 uL |
| 99874: DNA Bead Mix | 36 mL |

### Storage and Handling

Upon receipt, store DNA Bead Mix, 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.

**Note:** Do not freeze DNA Bead Mix.

### 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™ 2.0 FFPE DNA Extraction Kits 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.

This kit uses magnetic beads for DNA isolation and may be adapted for multiwell plate format. We also offer the CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kit for spin column-based DNA purification (see Related Products).

### Evaluating FFPE DNA Extraction

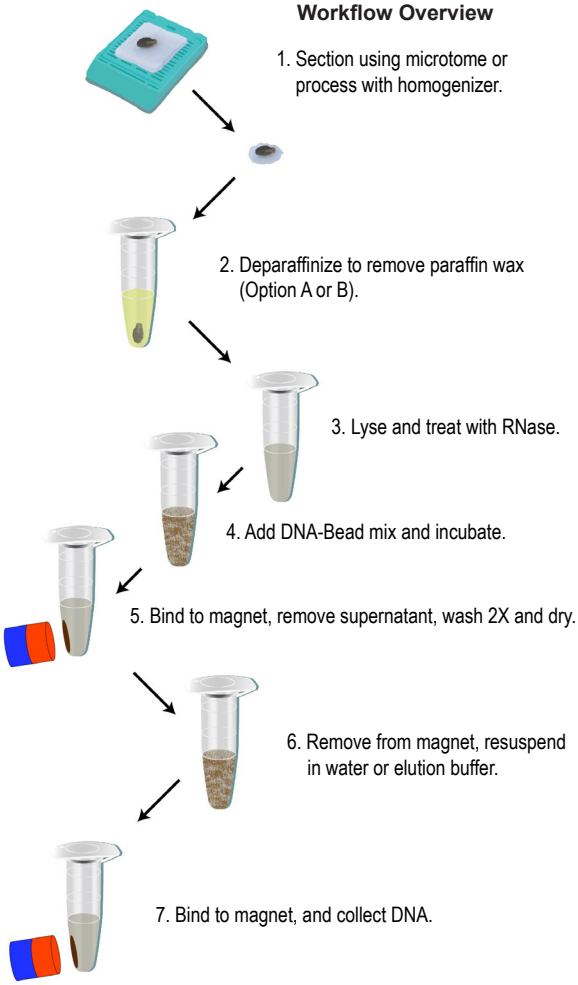
The following techniques may be used to evaluate the quality and quantity of your FFPE-derived DNA after extraction.

- **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. Consider using 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, proteins, and other impurities are removed from the DNA. The DNA is first bound to paramagnetic beads in the presence of crowding agents, then washed using 80% ethanol. Pure DNA is finally eluted using water or a low-salt buffer.



## Automated Extraction of DNA

While the protocol below describes manual extraction of DNA in 1.5 mL tubes, the magnetic bead isolation steps may be adapted for automated processing using a general-purpose liquid handler in 96-well format. It is recommended that all steps up to Protocol Section 3 be performed manually.

## Materials required but not supplied

- A microtome for tissue sectioning
- Optional: Xylenes may be used in place of the provided Dewaxing Solution (see Option B in protocol)
- Freshly prepared 80% ethanol in water (molecular biology grade)
- Heat blocks set to 37°C, 56°C, and 80°C
- An ice-filled container appropriate for microcentrifuge tubes
- 1.5 mL microcentrifuge tubes (Eppendorf LoBind® tubes recommended)
- Microcentrifuge (12,000 x g minimum)
- Magnetic rack (for 1.5 mL tubes, or a magnet plate if isolations are performed in 96-well format).
- Elution solution: We recommend using 10 mM Tris pH 8 or TE buffer (10 mM Tris, 1 mM EDTA pH 8). Nuclease-free water may also be used.

## Before You Begin

### 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 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.

Warm the DNA Bead Mix to room temperature before use.

## Detailed DNA Isolation Protocol

### Option A (recommended): Deparaffinization using included reagent

This recommended procedure uses the convenient Dewaxing Solution provided in the kit. Unlike xylenes, the Dewaxing Solution efficiently removes paraffin without a wash step and does not need to be handled in a fume hood. An alternative protocol using xylenes is also provided below (Option B).

**Note**: Additional Dewaxing Solution may be purchased separately for dissolving larger amounts of paraffin. However, we do not recommend using excess input tissue because it may not be efficiently lysed in subsequent steps.

A1. Place 1 to 4 sections into a 1.5 mL microcentrifuge tube.

A2. Add 500 uL of Dewaxing Solution to the tube.

A3. Invert the tube several times to mix until the wax has dissolved.

Optional: If solid (white) wax appears to still be present, you may heat the tube for 1-5 minutes or longer in a heat block at 37°C or 56°C until the wax is transparent. Note that the tissue will remain solid at this stage.

A4. Centrifuge briefly to collect the tissue at the bottom of the tube.

A5. Carefully pipette off the Dewaxing Solution from the tissue. Use a fine tip to carefully remove as much residual solution from the bottom of the tube as you can without disturbing the tissue pellet.

A6. Place the tube with lid open in a heat block at 37°C for 10 minutes to dry the tissue.

A7. 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. Proceed to step 1.

### 1. Uncrosslinking and lysis

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

1.2 Add 20 uL of Proteinase K to each tube. Mix the solution briefly by pipetting up and down, and then centrifuge at 16,000 x g for 30 seconds.

1.3 Incubate the tubes in a heat block at 56°C for 1 hour.

1.4 Move the tubes to a heat block at 80°C and incubate for 4 hours.

1.5 Place the tubes on ice for 1 minute.

1.6 Centrifuge briefly to collect contents at the bottom of the tubes.

1.7 Using a pipette, carefully transfer as much of the supernatant (containing DNA) as possible to clean tubes, without disturbing the pellets. Discard the pellets.

### 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.

2.1 Add 10 uL of RNase A to each tube of supernatant from step 1.7 and incubate at room temperature for 15 minutes.

### 3. DNA isolation

3.1 Warm the DNA Bead Mix to room temperature and mix well by inverting until solution appears homogeneous. **Important:** Do not vortex DNA Bead Mix.

3.2 To each tube, add 320 uL of DNA Bead Mix. Mix well by pipetting up and down several times until the mixture is homogeneous.

3.3 Let the tubes stand for 10 minutes at room temperature to allow DNA to bind to the beads.

3.4 Place the tubes on a magnetic rack and let stand for 5 minutes or until all beads are bound to the magnet.

3.5 Remove the supernatant, being careful not to aspirate any beads.

3.6 Leaving the tubes on the magnet, wash the beads by adding 1 mL of 80% ethanol to each tube. Dispense the wash against the side of the tube away from the beads, so that the wash solution is not dispensed directly onto the beads.

3.7 Incubate the beads with the wash solution at room temperature for at least 30 seconds.

3.8 Remove and discard the wash solution, being careful not to aspirate any beads.

3.9 Wash the beads again by repeating steps 3.6 to 3.8.

3.10 Dry the beads at room temperature for 3 to 5 minutes or until all ethanol has evaporated. Do not over dry the beads, as this may affect DNA recovery.

3.11 Remove the tubes from the magnet.

3.12 Elute the DNA by adding 50 uL of Tris or Tris-EDTA buffer (pH 8), or nuclease-free water. Mix well by pipetting up and down until the beads are fully resuspended.

3.13 Incubate the tubes for 10 minutes at 37°C.

3.14 Briefly centrifuge the tubes to collect contents at the bottom. Place the tubes on the magnetic rack and let stand for 1 to 2 minutes or until all beads are bound to the magnet.

3.15 Transfer the eluate to a clean tube.

3.16 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 optional 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. Please note that no more than 4 sections (10 um each) should be used for each extraction. Using too much tissue can lead to incomplete digestion and reduced yields. |
| 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. As cores are not processed using a microtome, sample digestion tends to be more difficult, and mechanical homogenization (e.g., steel beads) is recommended if incomplete digestion is observed. |
| Which deparaffinization method do you recommend? | CELLDATA FFPE Kits include a recommended Dewaxing Solution. The Dewaxing Solution removes wax without a separate wash step, is less hazardous than xylenes, and does not need to be handled in a fume hood. |
| 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.<br>An in-depth discussion of this issue is found in Dietrich et al. (2013), PLoS ONE 8(10): e77771. |

## Related Products

| Cat. No. | Product |
|---|---|
| 31030 | DNA Gel Extraction Kit |
| CD507 | CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kit |
| CD506 | CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit |
| CD508 | CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination Kit |
| 31028 | AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit |
| 31060 | AccuBlue® NextGen dsDNA Quantitation Kit |
| 31066 | AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®) |
| 31073 | AccuBlue® Broad Range RNA Quantitation Kit |
| 41003 | GelRed® Nucleic Acid Gel Stain, 10,000X in Water |
| 41005 | GelGreen® Nucleic Acid Gel Stain, 10,000X in Water |
| 41029 | GelRed® Agarose LE |
| 41030 | GelGreen® Agarose LE |
| 41020 | 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 |

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.