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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Product Information

CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kit

Catalog Number: CD507 (50 preps)

Kit Contents

Component	Size
99876-30ML Dewaxing Solution	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

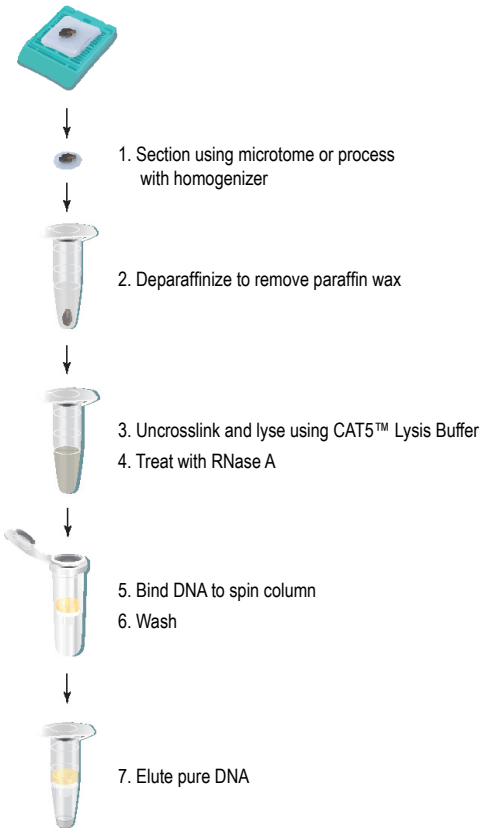
The following techniques may be used to evaluate the quality and quantity of your FFPE-derived DNA after extraction.

- **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 Dewaxing Solution (see Option B in protocol)
- Ethanol (200 proof, 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)
- 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

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 the tube containing deparaffinized tissue 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.

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

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

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

1.5 Place the tube on ice for 1 minute.

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

1.7 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.

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 1.7 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 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. 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?	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. 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
CD506	CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit
CD508	CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination 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.

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