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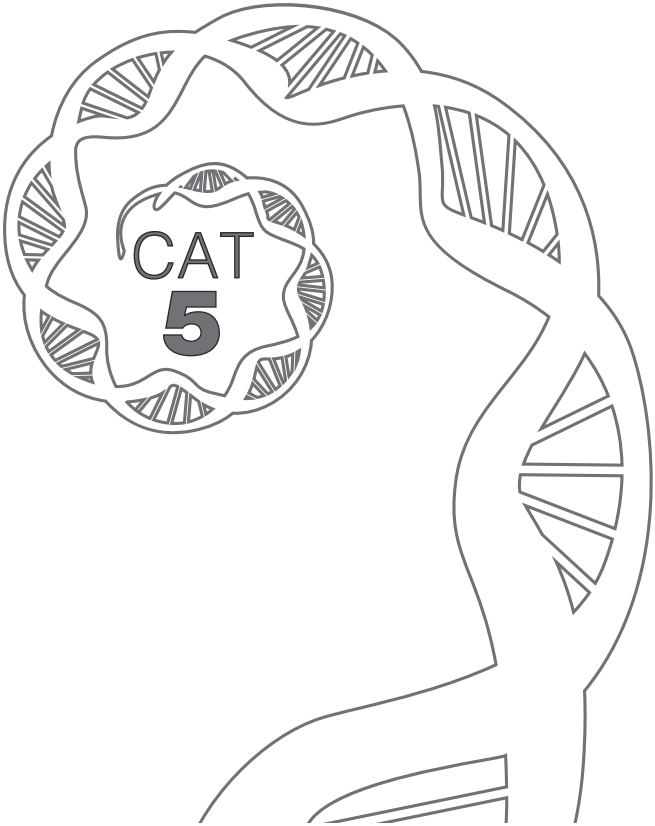
DNAstorm™ MagBead FFPE

DNA Isolation Kit for FFPE Tissue Samples

Kit Manual

96 extractions (CD505-96)



rev. 1.0

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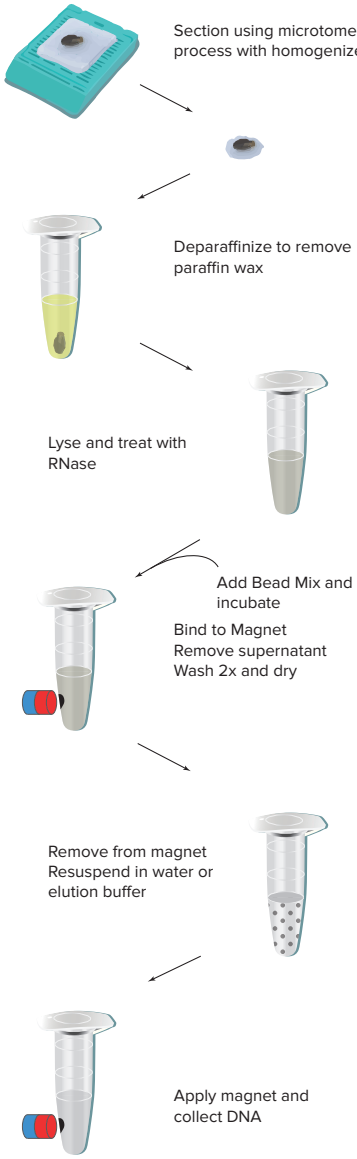
DNAstorm™ MagBead Kit for Isolation of DNA from FFPE Tissues

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™ MagBead 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 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.

Note regarding automated extraction of DNA

While the protocol below describes manual extraction of DNA in 1.5mL tubes, the DNAstorm™ kit can be easily adapted for automated extraction using a general purpose liquid handler and in 96-well format. It is recommended that steps A1-A8 and 1-7 be performed manually. Please contact support for additional information and guidelines regarding high-throughput use.

Kit Contents

The following reagents are included with the DNAstorm™ MagBead kit:

Reagent	Volume
 CAT5™ Lysis Buffer	25 mL
 Deparaffinization Reagent	60 mL
 DNA Bead Mix	36 mL. Warm to room temperature before use. Invert to mix. **Do not vortex.**
 Proteinase K	2.4 mL (2 vials, 1.2 mL each)
 RNase A	1.2 mL

Storage Conditions:

- Store DNA Bead Mix at 2-8˚C and protect from light. All other 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).
- Freshly prepared 80% ethanol (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).
- Magnetic rack (for 1.5 mL tubes, or a magnet plate if extractions are performed in 96-well format).
- DNA Bead Mix: warm to room temperature and mix well before use. Invert to mix. **Do not vortex.**
- 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™ MagBead FFPE 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

Deparaffinization

A1. Place 1 to 4 sections in a 1.5 mL Eppendorf tube.

A2. Add 500 µL of Deparaffinization Reagent.

A3. Vortex or shake for 10 seconds. If necessary, 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.

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 quickly spin down.
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. 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 8.

7. Add 10 µL of RNase A and invert tubes gently to mix. Briefly spin down and incubate at room temperature for 15 minutes.

DNA Isolation

8. To each tube, add 320 µL of DNA Bead Mix. Mix well by pipetting up and down several times until the mixture is homogeneous. *Note:* Before use warm DNA Bead Mix to room temperature and mix well by inverting until solution appears homogeneous. Do not vortex DNA Bead Mix.
9. Let mixture stand 10 minutes at room temperature to allow DNA to bind to beads.
10. Place tubes on magnetic rack and let stand 5 minutes or until all beads are bound.
11. Remove supernatant, being careful not to aspirate any beads.
12. Leaving tubes on magnet, wash beads by adding 1 mL of 80% ethanol. When adding ethanol wash do not dispense directly onto beads.
13. Incubate with ethanol wash solution at room temperature for at least 30 seconds.
14. Remove and discard ethanol wash, being careful not to aspirate any beads.
15. Wash beads again by repeating steps 12 to 14.
16. Dry beads at room temperature for 3 to 5 minutes or until all ethanol has evaporated. Be careful not to over dry the beads, as this may affect DNA recovery.
17. Remove tubes from magnet.
18. Elute DNA by adding 50 µL 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.

19. Incubate tubes for 10 minutes at 37˚C.
20. Quickly spin down tubes. Place tubes on magnetic rack and let stand 1 to 2 minutes or until all beads are bound.
21. Transfer eluate to a clean tube.
22. 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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