Product Information

CELLDATA RNAstorm™ 2.0 MagBead FFPE RNA Extraction Kit, 96 preps

Catalog Number: CD510-96

Kit Contents

Component	Size
99876-30ML: Dewaxing Solution	2 x 30 mL
99861: RNAstorm™ FFPE CAT5™ Reagent	2 x 5 mL
99862: RNAstorm™ FFPE Lysis Buffer	2 x 5 mL
99866: RNAstorm™ FFPE Protease	2 x 600 uL
99865: DNase Buffer	2 x 5 mL
99867-600U: DNase I (lyophilized)	2 x 600 U
99883: RNA Bead Buffer	12 mL
99884: RNA MagBeads	36 mL

Storage and Handling

Upon receipt, store RNA MagBeads, RNA Bead Buffer, FFPE Protease, and DNase I at 2-8°C. Store other kit components at room temperature. After reconstitution, DNase I solution should be stored at -20°C. Kit components are stable for at least 9 months from date of receipt when stored as recommended.

Note: Do not freeze RNA MagBeads.

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 such as RNA. In contrast, the catalytic technology used in the CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kits greatly accelerates the removal of formaldehyde damage and allows the use of milder conditions, resulting in markedly improved recovery of amplifiable RNA. Compared to other methods, this greatly enhances the chances of success in recovering higher 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 RNA isolation and may be adapted for a multiwell plate format. We also offer the CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit for spin column-based RNA purification, and CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kits for column or bead-based isolation (see Related Products).

Evaluating FFPE RNA Extraction

The following techniques may be used to evaluate the quality and quantity of your FFPE-derived RNA after extraction (see FAQ section for more).

Quantity/Concentration: The RNA 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 RNA per sample. Measurements can be obtained using quantitative RT-PCR. Note that UV/Vis spectroscopy (e.g., NanoDrop®) may also be used but is particularly susceptible to contaminants such as cellular debris, proteins, salts, and detergents which absorb in the 200-280 nm range.

RNA Integrity: Agarose gel electrophoresis or capillary gel electrophoresis, such as the Agilent Bioanalyzer®, expressed as a RIN number or DV200 percentage can be used to evaluate RNA integrity. Note that FFPE RNA will have a lower molecular weight profile compared to RNA from fresh samples, and will not show intact rRNA bands.

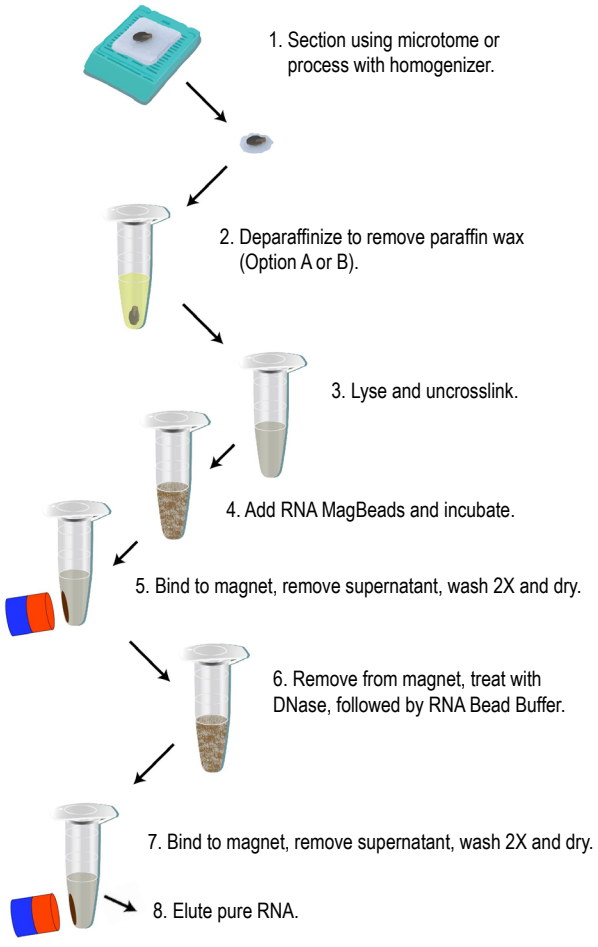
Amplifiability: The standard method is quantitative RT-PCR expressed as a Ct number or as a relative or absolute amount of RNA.

Protocol Outline

The RNAstorm™ FFPE RNA 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 RNA from other cellular components and to remove formaldehyde-induced modifications.
- **Nucleic acid capture:** Nucleic acids are bound to paramagnetic beads in the presence of crowding agents, then washed using 80% ethanol.
- **DNase I treatment (on beads):** Contaminating genomic DNA is degraded using DNase I. This step is optional but highly recommended.
- **RNA isolation:** RNA is re-bound to paramagnetic beads in the presence of crowding agents, then washed using 80% ethanol. Pure RNA is finally eluted using water.

Workflow Overview



Automated RNA Extraction

While the protocol below describes manual extraction of RNA 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 through protocol section 1 be performed manually.

Materials required but not supplied

- 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 and 72°C
- An ice-filled container appropriate for micro-centrifuge 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).
- RNase-free water for DNase I reconstitution and final RNA elution step

Before You Begin

Prepare the following:

- DNase I: Reconstitute the lyophilized DNase I by adding 120 uL of RNase-free water. Using a pipette, mix gently to ensure the DNase is fully reconstituted (do not vortex, which can denature DNase). Briefly centrifuge the tube to collect contents. Store in aliquots at -20°C and avoid freeze/thaw cycles.
- Prepare fresh 80% ethanol in water using molecular grade reagents. You will need at least 4 mL of 80% ethanol per sample; we recommend preparing 20% excess volume to compensate for loss during pipetting.

Prepare the tissue

The RNAstorm™ 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 RNA 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 72°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.

Note: Residual Dewaxing Solution will not interfere with subsequent extraction and does not need to be completely removed or dried.

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 the desired number of sections in a 1.5 mL microcentrifuge tube.

B2. In a fume hood, add 1 mL of xylenes and close the tube lid. Invert the tube several times to mix, 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 invert the tube to mix. 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 samples.

B8. Proceed to step 1.

1. Uncrosslinking and lysis

1.1 Add 80 uL of RNAstorm™ FFPE CAT5™ Reagent 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 the RNAstorm™ FFPE CAT5™ Reagent.

1.2 Incubate the tube containing tissue in a heat block at 72°C for 30 minutes, then place on ice for 1 minute.

1.3 Add 80 uL of RNAstorm™ FFPE Lysis Buffer to the tube, and then add 10 uL of RNAstorm™ FFPE Protease. Invert the tube gently several times to mix, then centrifuge briefly to collect contents at bottom of the tube.

1.4 Incubate the tube in a heat block at 72°C for 2 hours.

1.5 Place the tube on ice for 3 minutes.

1.6 Centrifuge the tube for 15 minutes at 16,000 x g. A pellet will form containing cellular debris, while the RNA will remain in solution. The pellet may not be visible, but this will not affect the yield and quality of the obtained RNA.

1.7 Using a pipette, carefully transfer as much of the supernatant as possible to a new tube, without disturbing the pellet.

2. Nucleic acid capture on beads

2.1 Warm the RNA MagBeads to room temperature and mix well by inverting or pipetting up and down until the suspension appears homogeneous.

Important: Do not vortex RNA MagBeads, because this may cause the beads to aggregate.

2.2 Add 310 uL of RNA MagBeads to each sample tube and mix well by pipetting up and down.

2.3 Let the tubes stand for 10 minutes at room temperature to allow nucleic acids to bind to the beads.

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

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

2.6 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.

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

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

2.9 Wash the beads again by repeating steps 2.6 to 2.8.

2.10 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 RNA recovery.

2.11 Proceed to step 3 (DNase treatment, recommended) or step 4 to elute RNA without DNase treatment.

3. DNase treatment (recommended)

Note: This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 4.1.

3.1 For each sample, mix 70 uL of DNase Buffer with 2 uL of reconstituted DNase I.

3.2 Remove the sample tubes from the magnet. Add 72 uL of the DNase I reaction mixture from the previous step to the beads and resuspend by pipetting up and down until the mixture is homogeneous.

3.3 Incubate the tubes at 37°C for 15 minutes.

3.4 Add 105 uL of RNA Bead Buffer to each tube and mix well by pipetting up and down until the mixture is homogeneous.

Note: RNA Bead Buffer is required for the RNA to bind to the beads after DNase treatment.

3.5 Let the tubes sit off the magnet for 10 minutes at room temperature.

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

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

3.8 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.

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

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

3.11 Wash the beads again by repeating steps 3.8 to 3.10.

3.12 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 RNA recovery.

4. RNA elution

4.1 Remove the tube from the magnet. Add 50 uL of RNase-free water to the beads and pipette up and down to mix until the mixture is homogeneous.

4.2 Let the tubes sit off the magnet for 10 minutes at room temperature.

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

4.4 Remove the supernatant, which contains eluted RNA, and transfer it to a clean tube, being careful not to aspirate any beads.

4.5 Eluted RNA should be stored at -80°C.

Frequently Asked Questions (FAQs)

Question	Answer
Is any contaminating genomic DNA present in the RNA obtained using the RNAstorm™ kit?	Contamination from genomic DNA is a big concern because it can interfere with downstream applications. The RNAstorm™ kit includes an optimized DNase digestion step which removes contaminating genomic DNA without significantly affecting RNA yield. While this step is optional, it is highly recommended.
How much RNA can I expect to obtain from an FFPE sample?	The biggest variable that affects the total amount of RNA 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 RNAstorm™ kit, and assuming at least reasonable sample quality, amounts greater than 1 ug can be obtained.
Can RNA obtained using the RNAstorm™ kit be used in RNA-Seq?	Yes. Good quality libraries can be obtained, providing that the RNA is of sufficiently high quality. For Illumina® sequencing, a DV200 of at least 30% is recommended, and samples should be used that provide at least 1 ug of RNA.
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. Also, 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.
What is the best way to quantitate RNA obtained from FFPE samples?	FFPE-derived RNA is much more challenging to quantitate accurately than RNA obtained from fresh samples. It is not enough to know the absolute amount of RNA that is present, but also whether the RNA will work in downstream applications, which depends on the following factors: • Fragment size distribution: A 5 ug sample (as measured by Qubit®) can be useless for RNA-Seq if it consists of fragments < 200 nt. • Chemical modification: For RNA obtained from formalin-fixed samples, various chemical adducts and crosslinks, including base modifications, base-base crosslinks, and base-protein crosslinks can make nucleic acid molecules inaccessible to enzymes and therefore inactive in downstream applications. • Contamination: Cellular debris, proteins, salts, and detergents used during purification can bias downstream assays. For example, UV/Vis-based methods such as NanoDrop® are particularly susceptible to contaminants which absorb in the 200-280 nm range. • Fluorescence-based methods such as Qubit® are liable to significant error. When working with low concentrations of DNA or RNA, dye-based detection may not be linear. One must also be mindful of contamination by genomic DNA in an RNA sample, because the dyes used for fluorescence quantitation are not entirely specific for FFPE-derived DNA or RNA. • Quantitative PCR is the preferred method for quantitation of heavily damaged and modified nucleic acids.

Frequently Asked Questions (FAQs), continued

Question	Answer
Should RIN numbers be used to determine quality of FFPE-derived RNA?	Although the RIN number can provide general information about the extent of sample fragmentation, it is not sensitive or predictable enough to be a useful indicator of downstream performance, especially for RNA-Seq. Very often, RIN numbers for FFPE-derived RNA will be between 2 and 3. Some of these samples will be useful for RNA-Seq, and others won't - the RIN will not tell you, however. A slightly better predictor of performance in RNA-Seq using Illumina® sequencing is the DV200, which represents the percentage of RNA fragments longer than 200 nucleotides. The DV200 is also calculated based on Bioanalyzer® data, but suffers from the same drawbacks as all Bioanalyzer®-based methods, specifically high variability.
What do I need to know when extracting RNA from FFPE samples?	• Avoid methods based on organic solvents (TRIzol™) • Avoid harsh chaotropic salts (i.e., guanidinium) • Avoid detergents which impact downstream quantitation by UV and/or Qubit® (e.g., Triton® X-100) • Do not rely on RIN to quantitate integrity of an FFPE-derived sample. Use DV200 instead. • Use a kit or method such as the RNAstorm™ kit that removes chemical modifications from formalin. Do not raise the temperature to 80°C or above. Even short times at this temperature will significantly lower integrity. • Be wary of Qubit® and NanoDrop® concentrations because of the possibility of contamination by organic molecules or DNA. • Use qPCR to quantitate your RNA, and always look carefully at melt curves to determine whether nonspecific amplification may have occurred.

Related Products

Cat. No.	Product
CD506	CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit, 50 preps
CD507	CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kit
CD508	CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination Kit
CD509-96	CELLDATA DNAstorm™ 2.0 MagBead FFPE DNA Extraction Kit, 96 preps
28001	ExoBrite™ EV Total RNA Isolation Kit
41032	EMBER500™ RNA Prestain Loading Dye
41044	EMBER™ Ultra RNA Gel Kit
31073	AccuBlue® Broad Range RNA Quantitation Kit
22028	RNase-X™ Decontamination Solution
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
31042	Forget-Me-Not™ qPCR Master Mix
31043	Forget-Me-Not™ Universal Probe Master Mix
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 RNA research and applications, including RNA extraction kits for fresh cells and FFPE tissues, RNA quantitation kits, and RNA gel stains.

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