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RNA*later*®-ICE Frozen Tissue Transition Solution

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IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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Product information

Product description

RNA*later*®-ICE is a novel reagent for transitioning frozen tissue to a state that can be readily processed by common homogenization methodologies to extract high quality RNA. It circumvents the need to grind frozen tissue into a powder before homogenization, and even makes it possible to further dissect tissues before homogenization without RNA loss or degradation.

Difficulties in homogenization of frozen tissue samples.

Many researchers rapidly freeze their tissue samples, usually by submersion in liquid nitrogen, to maintain their RNA integrity so that they can be stored for RNA extraction at a later time. Once frozen, samples must remain at –70°C or colder for maximum protection of the RNA. The difficulty faced by many researchers is that frozen tissue is so brittle and hard that it is at odds with the standard processing protocols used for RNA extraction. Thawing

the tissue for homogenization, however, clearly results in RNA degradation and alteration of the RNA profile. Very small pieces of frozen tissue can sometimes be dropped into lysis solution and immediately homogenized for RNA isolation, but the timing of the procedure must be perfect to avoid RNA degradation. Larger frozen tissue samples cannot be disrupted quickly enough to avoid thawing, and thus RNA degradation, if they are dropped into a homogenizer containing lysis solution for disruption. To avoid this, frozen tissue is usually pulverized into a powder with liquid nitrogen or dry ice to keep it cold, and the powdered tissue is added directly to a lysis solution. The mixture is then immediately homogenized. This procedure is not only messy and laborious, but sample is inevitably lost during the pulverization process and/or the transfer to a homogenizer containing lysis solution. Also, frozen tissue powder often forms clumps upon contact with the >0°C lysis solution, resulting in localized thawing and RNA loss.

RNA*later*®-ICE transitions frozen tissue to a consistency that is compatible with homogenization

Soaking frozen tissue samples overnight in RNA*later*®-ICE at –20°C eliminates the need to pulverize frozen tissue samples, because soaking in RNA*later*®-ICE changes the physical state of the tissue from brittle to pliable at –20°C, minimizing RNA degradation. Once permeated with RNA*later*®-ICE, tissue samples can be processed using the same techniques as for fresh tissue. Tissue treated with RNA*later*®-ICE is even easier to work with than fresh tissue, because the need to work quickly to avoid RNA degradation is relaxed. Once tissue has been transitioned in RNA*later*®-ICE, it can be removed from the solution, and left at room temperature for up to 30 minutes without affecting the RNA. Also, tissues permeated with RNA*later*®-ICE can be stored at –20°C long-term if desired.

RNA*later*®-ICE contains a blue dye that is visible in samples that have been permeated with the solution; this makes it easy to identify samples that have been treated with RNA*later*®-ICE.

Compatibility and requirements for using RNA*later*®-ICE

RNA*later*®-ICE is for use only with frozen tissue

RNA*later*®-ICE is designed for use with frozen samples only.

What sample types can be transitioned in RNA*later*®-ICE?

RNA*later*®-ICE was designed to work with frozen tissues primarily from vertebrate species. It has been tested extensively with brain, heart, kidney, spleen, liver, pancreas, lung and thymus. Although intended for animal tissue, RNA*later*®-ICE is also effective for frozen cultured mammalian cell pellets, and frozen white blood cell pellets.

Sample size and shape

Tissue samples must be 0.5 cm or smaller in the smallest dimension. As long as samples are less than 0.5 cm thick, their size in length or width is not important.

Cell pellets should have a maximum thickness of 0.5 cm.

Will RNA*later*®-ICE work with my RNA isolation kit?

RNA*later*®-ICE is compatible with most RNA isolation methods. Specifically, we have isolated RNA from samples in RNA*later*®-ICE with most of the Ambion® RNA isolation kits and reagents, including:

- RNAwiz™ (available only in Japan) and TRI Reagent®: one-step disruption/separation reagents
- RNAqueous® Kits: phenol-free, glass fiber filter binding
- RiboPure™ Kits: combination organic and solid phase RNA isolation procedure for exceptionally pure RNA
- MagMAX™ Kits: magnetic bead-based RNA isolation
- *mir*Vana™ miRNA Isolation Kits: designed for recovery of all RNA species, including small RNAs
- ToTALLY RNA™: guanidinium isothiocyanate disruption, acid phenol extraction

Genomic DNA from RNA*later*®-ICE stored samples

For more information, go to **http://www.lifetechnologies.com/us/en/home/references/protocols/nucleic-acid-purification-and-analysis/rna-protocol/genomic-dna-preparation-from-rnalater-preserved-tissues.html**

Transitioning tissue from –70°C or colder to –20°C

1. Prechill a minimum of 10 volumes of RNA*later*®-ICE at –70°C or –80°C in a polypropylene tube.
   - The tube should be large enough for the tissue to move freely when inverted; this is important for uniform permeation with RNA*later*®-ICE.
   - Prechill 10 volumes of RNA*later*®-ICE compared to the sample mass. For example, prechill 2.5 mL of RNA*later*®-ICE for a 250 mg sample.
2. Combine *frozen* tissue with a minimum of 10 volumes of prechilled RNA*later*®-ICE.

Type of tissue	Details
Frozen tissue pieces (no larger than 0.5 cm in the smallest dimension)	Place frozen tissue into the RNA*later*®-ICE. It is important that the RNA*later*®-ICE stays cold, so work quickly. If you are working with many samples at the same time, you may want to set up the tubes of RNA*later*®-ICE in a container of dry ice.
Frozen cell pellets	Pipet RNA*later*®-ICE into the tube, then tightly cap the tube and invert several times to mix. Cell pellets typically do not detach from the bottom of the tube - this is not problematic.

3. Soak the tissue in RNA*later*®-ICE at –20°C for at least 16 hours.

   Tissue samples may be stored in RNA*later*®-ICE at –20°C for up to 6 months with no loss of RNA integrity. After equilibration in RNA*later*®-ICE, samples are also resistant to RNA degradation when temporarily subjected to higher temperatures (that is, freezer failure).

Storage of samples treated with RNA*later*®-ICE

- Samples transitioned to –20°C in RNA*later*®-ICE should be left in the RNA*later*®-ICE, and maintained at –20°C or colder.
- Once the initial overnight soak at –20°C is complete, the RNA in the sample will remain intact even if the tissue is left for up to ~30 minutes at room temperature, and as long as overnight at 4°C.

RNA isolation from samples treated with RNA*later*®-ICE

1. Remove tissue from RNA*later*®-ICE.

Type of tissue	Detailed procedure
Tissue pieces	Remove tissue pieces from RNA*later*®-ICE and place them into empty homogenization tubes. Although we recommend working quickly, tissues are stable for at least 30 minutes at room temperature after they are removed from RNA*later*®-ICE. This allows for time to further dissect and weigh samples if desired. Treated samples can be returned to –20°C for further storage after a portion is removed for RNA isolation.
Cell pellets	Centrifuge ~1 min at 10,000 × *g* at 4°C to separate cells (for example, white blood cells or cultured mammalian cells) from RNA*later*®-ICE. Cells can withstand centrifugation at much higher forces after storage in RNA*later*®-ICE.

2. Proceed with RNA isolation.

   As for any RNA isolation procedure, be sure to homogenize tissue immediately after placing it in the lysis solution or one-step reagent.

RNA*later*®-ICE specifications

Kit contents

Cat. no.	Size
AM7030	25 mL
4427575	10 x 25 mL

Storage and stability

Store RNA*later*®-ICE at room temperature. Keep container tightly closed when not in use, evaporation may occur.

Appendix A Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs), and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: **www.cdc.gov/biosafety**/publications/bmbl5/BMBL.pdf
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf.

Documentation and support

Revision history

Revision	Date	Description
D	November 2014	Corrected URL for genomic DNA isolation procedure. Legal and style updates.
C	March 2011	Baseline for this revision history.

Customer and technical support

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- Worldwide contact telephone numbers
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  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs, also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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