RNA*later*™

Tissue Collection: RNA Stabilization Solution

Catalog #7020 (100 ml), 7024 (250 ml), 7021 (500 ml)

Protocol

Ambion®
THE RNA COMPANY™

A. Product Description

RNA*later*™ is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissue to stabilize and protect cellular RNA in situ in ***unfrozen*** specimens. Tissue pieces are harvested and immediately submerged in RNA*later* for storage without jeopardizing the quality or quantity of RNA. RNA*later* eliminates the need to immediately process tissue specimens or to freeze samples in liquid nitrogen for later processing. The figures below show 2 common experiments using RNA isolated from RNA*later*-preserved samples.

RNA*later* preserves RNA in tissues for up to 1 day at 37°C, 1 week at 25°C, and 1 month or more at 4°C. Tissues can also be stored at –20°C or at –80°C long-term.

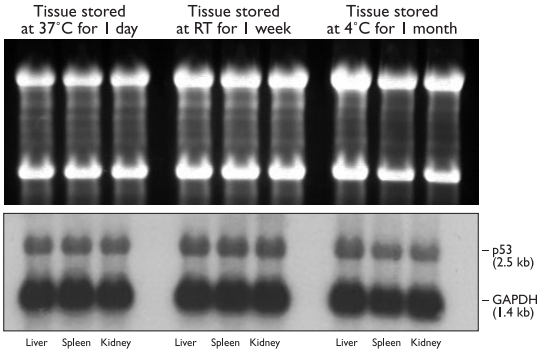

Figure 1. RNA from Tissue Stored in RNA*later*

RNA was extracted from mouse tissues stored in RNA*later* as shown. The top panel shows an ethidium bromide-stained denaturing agarose gel; the bottom panel shows a Northern blot.

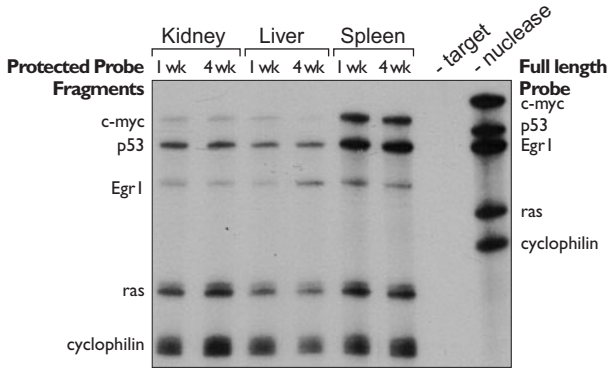

Figure 2. mRNA profiles of mouse tissues stored in RNA*later*

The indicated mouse tissues were stored in RNA*later* for 1 or 4 weeks at 4°C. RNA was isolated from each tissue and analysed using Ambion's RPA III™ kit. 10 µg of RNA was hybridized with a mixture of $5x10^4$ cpm of each of 5 antisense probes. The gel was exposed to film for 4 hours at –80°C with an intensifying screen.

Storage and Stability

Store RNA*later* at room temperature. It is guaranteed for 6 months from the date received.

If any precipitation of RNA*later* is seen, heat the solution to 37°C and agitate to redissolve it.

What materials have been tested in RNA*later*?

RNA*later* has been extensively tested on tissues from several vertebrate species. These include brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung and thymus. RNA*later* is also effective for *E. Coli*, *Drosophila*, tissue culture cells, white blood cells, and some plants.

Will RNA*later* Work with my RNA Isolation Kit?

RNA*later* is compatible with most RNA isolation methods. Specifically, we have used RNA*later*-preserved samples with TRI Reagent®*, and all of Ambion's RNA isolation kits and reagents, including: RNAw z™ (one-step disruption/separation reagent), TōTALLY RNA™ (guanidinium isothiocynate disruption, acid phenol extraction), RNAqueous™ (phenol-free, glass fiber filter binding), and Poly(A)Pure™ (direct isolation of poly(A) RNA from guanidinium lysate).

* TRI Reagent, and TRIzol are registered trademarks of Molecular Research Center Inc.

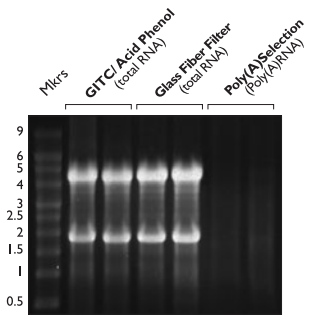

Figure 3. RNA isolated from tissue stored in RNA*later* using different isolation methods

Whole mouse hearts and livers were dissected, and placed in RNA*later*, in which they were stored for 3 days at 4°C. RNA was isolated from equal mass amounts of each tissue using the indicated Ambion kits. RNA (5 µg) was run on denaturing agarose, stained with ethidium bromide.

Can Genomic DNA be Obtained from RNA*later*-Stored Samples?

Yes, call Technical Service and request a protocol.

B. How to use RNA*later*

Use RNA*later* with fresh tissue only, do not freeze tissue before immersion in RNA*later*. Simply cut tissue samples to a maximum thickness of 0.5 cm in any 1 dimension, as long as samples are ≤0.5 cm thick, their size of the other dimensions is not important. Place the fresh tissue in 5 volumes of RNA*later,* and store as indicated for the desired temperature.

Animal Tissue

RNA*later* does not dissolve or disrupt the structure of tissue samples, thus tissue that has been equilibrated in RNA*later* can be removed from the solution, sectioned into smaller pieces, and returned to RNA*later* if desired. Small organs such as rat liver, kidney and spleen can be stored in RNA*later* whole.

Plant Tissue

Many plant tissues can be simply submerged in 5 volumes of RNA*later* for storage. We have successfully isolated intact RNA from tobacco leaf explants, entire arabidopsis and alfalfa seedlings, and from potato shoot tips. Plant tissues that have natural barriers to diffusion such as waxy coatings on leaves will probably require disruption to allow RNA*later* access to the tissue.

Tissue Culture Cells

Pellet cells according to the protocol followed by your laboratory. Wash to remove the culture medium (e.g. with PBS). Resuspend the cells in a small volume of PBS, then add 5 to 10 volumes RNA*later*.

White Blood Cells

White blood cells can be effectively preserved in RNA*later* if they are separated from the red blood cells and sera and treated as tissue culture cells. RNA*later* is not recommended for preserving RNA in whole blood, plasma, or sera. Because of their high protein content, these fluids will form an insoluble precipitate if they are mixed with RNA*later*.

Bacteria

RNA*later* is bacteriostatic; although bacteria do not grow in RNA*later,* the cells remain intact. *E. coli* stored in RNA*later* for 1 month at 4°C are intact and yield undegraded RNA.

C. Storage of Samples in RNA*later*

Storage at –80°C

Recommended for archival storage. Incubate samples at 4°C overnight, then remove them from RNA*later* before storage at –80°C. For tisssue culture cells, do not remove the RNA*later*, simply freeze the whole solution. The cell types we have tested do not lyse when frozen at –80°C in RNA*later*. Samples can subsequently be thawed at room temperature and refrozen without affecting the amount or the integrity of the recoverable RNA.

Storage at –20°C

Recommended for archival storage. Incubate samples at 4°C overnight, then transfer to –20°C. Samples will not freeze at –20°C, but crystals may form in the storage buffer; this will not affect subsequent RNA isolation. Samples can subsequently be thawed at room temperature and refrozen without affecting the amount or the integrity of the recoverable RNA.

Storage at 4°C

Ambion sees no evidence of RNA degradation in samples stored at 4°C for up to 1 month.

If Refrigeration is not Possible:

Place the samples in as cool an environment as possible. If ambient temperature is above 25°C, incubate samples in RNA*later* on ice for a few hours if possible before storing at ambient temperature.

Storage at 25°C

RNA isolated from samples stored at 25°C for one week is intact. In our experience, RNA from samples stored at 25°C for two weeks appears slightly degraded (marginally acceptable for northern analysis, but still of sufficient quality for nuclease protection assay or RT-PCR analysis).

Storage at 37°C

RNA isolated from samples stored at 37°C is intact after a 24 hour incubation, but is partially degraded after a 3 day incubation.

D. RNA Isolation from Material in RNA*later*

1. Removing Samples from RNA*later*

RNA*later* can be discarded down the sink with running water.

Tissue

Tissues that have been stored in RNA*later* should be removed from the storage solution with sterile forceps, and submerged in RNA isolation lysis solution. Tissue homogenization should be rapid once the tissue is in lysis/denaturation solution.

Cells

There are two options for isolating RNA from cells stored in RNA*later*, the RNA*later* can be removed, or the RNA can be extracted from the mixture of cells and RNA*later*.

- Removal of RNA*later*

  Our experience is that cells become much less fragile when stored in RNA*later* and can be centrifuged at high speed without lysis. We have successfully centrifuged cells at 5000 x g without loss. Since different cells may respond differently to this force, we suggest you try pelleting a non-valuable sample first to confirm that you can recover your cells this way. An alternative is to dilute the RNA*later* by 50% immediately before centrifugation with cold PBS (or other buffered solution) in order to reduce the density of the solution.

- RNA extraction from cells in RNA*later*

  Alternatively, we have used one-step disruption/extraction solutions (e.g. RNAwiz™, and TRI Reagent) to purify RNA from cells that have not been removed from RNA*later*. This can be done by adding ten volumes of the one-step solution to the cell mixture, and proceeding normally. When Ambion's RNAwiz™ is used in this way, it may be necessary to dilute the aqueous phase before the RNA precipitation step, see below for more information.

2. Tips for RNA Isolation

Glass fiber-based extraction

Using glass fiber filter-based RNA isolation kits, it may be necessary to use a centrifuge to push lysates through the filter as opposed to using a vacuum manifold.

One-step disruption/extraction solutions

When using one-step RNA isolation products such as TRIzol® (or TRI Reagent) on RNA*later*-preserved samples, occasionally the aqueous phase is cloudy. If this occurs, simply continue the procedure, following the manufacturer's instructions. Cloudiness of the aqueous phase does not affect the quantity or quality of the RNA obtained.

With Ambion's RNAwiz™, there may be a problem getting the aqueous phase to mix with isopropanol at the precipitation step because of RNA*later* carryover. If this occurs, simply add a mixture of 50% water, 50% isopropanol until the solution becomes clear and the two phases mix. The amount of water/isopropanol required will depend on how much RNA*later* was carried over; if the sample was mostly RNA*later*, as much as an equal volume may be needed.

E. RNA*later* Specifications

Quality Assurance:

RNA*later* undergoes quality assurance testing to verify that its composition is invariant from lot to lot.

Safety:

This product is a proprietary solution whose chemical, physical, and toxicological properties have not been thoroughly investigated. See the following MSDS for more information.

F. RNA*later*™ Material Safety Data Sheet

Physical data	
Appearance and odor	clear liquid, slightly viscous
Boiling point	n/a
Solubility in H_2O	soluble
Fire and explosion hazard data	
Flash point	n/a
Flammable limits in air	n/a
Extinguishing media	water, CO_2, foam, dry chemical (Use any means suitable for extinguishing surrounding fire)
Special fire fighting	Wear self-contained breathing apparatus and protective clothing.
Fire/explosion hazards	Irritating and/or toxic gases or fumes may be generated by thermal decomposition or combustion.
Health hazard data	
Effects of overexposure	Acute overexposure may cause irritation to eyes, skin, and respiratory tract.
Emergency first aid	Flush affected area with copious amounts of water. Irrigate eyes and skin for ≥15 minutes. Contact physician if irritation occurs due to salt content.

Reactivity data	
Stability	stable
Incompatibility	n/a
Haz. Decomp. Products	n/a
Hazardous Polymerization	n/a

Spill or leak procedures	
If released or spilled	Ventilate area. Absorb spill with inert material. Place in container with a lid. Wash spill area after cleanup.
Waste disposal method	Dispose of according to federal, local and state regulations.

Special protection and precaution information	
Respiratory protection	Not expected to require personal respirator usage. (Use NIOSH approved respirator if necessary)
Ventilation	Not expected to require special ventilation
Precautionary labeling	none
Handling and storage considerations	Laboratory aprons and gloves. Do not store in aluminum or copper containers. Keep tightly closed in a cool, dry place.