RNA*later*®

Tissue Collection: RNA Stabilization Solution

Cat #7020 (100 ml), #7024 (250 ml), #7021 (500 ml), #7022 (50 x 1.5 ml), #7023 (20 x 5 ml)

A. Product Description

RNA*later*® is an aqueous, nontoxic, tissue storage reagent that rapidly permeates most tissues to stabilize and protect RNA in fresh specimens. RNA*later* eliminates the need to immediately process or freeze samples; the specimen can simply be submerged in RNA*later* and stored for analysis at a later date.

Samples in RNA*later* can be stored for extended periods under conditions where RNA degradation would normally take place rapidly (Figure 1). Tissues can be stored indefinitely in RNA*later* at –20°C or below.

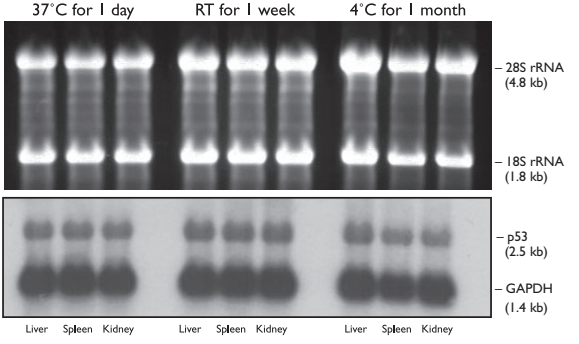

Figure 1. RNA from Tissue Stored in RNA*later*.

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

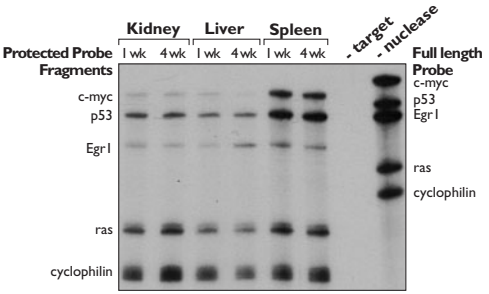

Figure 2. mRNA Profiles of Mouse Tissues Stored in RNA*later*

Mouse tissues were stored in RNA*later* for 1 or 4 weeks at 4°C. RNA was isolated from each tissue and analyzed using Ambion's RPA III™ Kit. The data demonstrate the stability of expression profiles in tissue stored in RNA*later*.

B. Product Guidelines

Storage and stability

- Store RNA*later* at room temperature. It is guaranteed for 6 months from the date of receipt, if properly stored.
- If any precipitation of RNA*later* is seen, heat the solution to 37°C and agitate to redissolve it.

Disposal of RNA*later*

RNA*later* can be safely discarded down the sink and flushed with water.

Materials compatible with RNA*later*

RNA*later* can be used for RNA preservation with most tissues, cultured cells, bacteria, and yeast. RNA*later* may not be effective in tissues that are poorly penetrated by the solution, such as waxy plant tissue and bone.

RNA*later* has been extensively tested with animal tissues, including brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung, and thymus. RNA*later* has also been proven effective for RNA preservation in *E. coli*, *Drosophila*, tissue culture

cells, white blood cells, and some plant tissues. Test results from additional samples can be found in Ambion's citation database at:

www.ambion.com/techlib/citations/index.php

RNA isolation from RNA*later*

RNA*later* is compatible with most RNA isolation methods. Samples from RNA*later* have been used successfully with TRI Reagent® (Cat #9738), and all of Ambion's RNA isolation kits and reagents, including: the TõTALLY RNA™ Kit, the PARIS™ Kit, the *mir*Vana™ miRNA Isolation Kit, and the RNAqueous® and Poly(A)Purist™ product families.

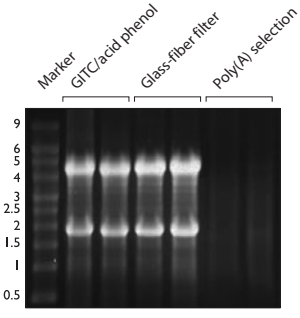

Figure 3. RNA isolated from tissue stored in RNA*later* using different isolation methods

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

Isolating genomic DNA from RNA*later*-stored samples

DNA can be isolated from RNA*later*-stored samples. See our website for a protocol at:

www.ambion.com/techlib/misc/genomicDNA_rnalater.html

Isolating protein from RNA*later*-stored samples

Proteins are also preserved in RNA*later*. RNA*later* will denature proteins; therefore, protein obtained from samples stored in RNA*later* will be suitable for applications such as Western blotting or 2D gel electrophoresis, but not for applications that require native protein.

C. Guidelines for Use of RNA*later*

- Use RNA*later* with ***fresh tissue only***; do not freeze tissues before immersion in RNA*later*.
- Before immersion in RNA*later*, cut large tissue samples to ≤0.5 cm in any single dimension.
- Place the fresh tissue in 5–10 volumes of RNA*later*.
- Most samples in RNA*later* can be stored at room temp for 1 week without compromising RNA quality, or at –20°C or –80°C indefinitely.
- Do not freeze samples in RNA*later* immediately; store at 4°C overnight (to allow RNA*later* to thoroughly penetrate the tissue), remove supernatant, then move to –20°C or –80°C for long-term storage.

NOTE

Ambion offers RNAlater®-ICE (Cat #7030) to recover tissues that have already been frozen. RNAlater-ICE renders frozen tissues pliant enough for homogenization while maintaining the low temperatures needed to protect the RNA from degradation.

Animal Tissue

RNA*later* does not disrupt the structure of tissues; 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 mouse liver, kidney and spleen can be stored whole in RNA*later*.

Plant Tissue

Plant tissues that have natural barriers to diffusion, such as waxy coatings on leaves, will often require disruption to allow RNA*later* access to the tissue. However, many plant tissues can simply be submerged in RNA*later* whole; we have successfully isolated intact RNA from tobacco leaf explants, entire *Arabidopsis* and alfalfa seedlings, and from potato shoot tips.

Tissue Culture Cells

Pellet cells according to the protocols followed by your laboratory. Remove supernatant and then add 5–10 volumes RNA*later*. The cells can be washed in PBS before resuspending in RNA*later*, if desired.

Blood and Plasma

White blood cells can be effectively preserved in RNA*later* when separated from the red blood cells and sera and treated as tissue culture cells. RNA*later* will preserve RNA in small amounts of anticoagulated whole blood, sera, and plasma; however, the procedure is more involved; see Ambion's RiboPure™ Blood Kit (Cat #1928) manual for specific instructions on use of RNA*later* with whole blood.

Yeast

Pellet up to 3 x 10^8 cells (centrifuge at 12,000 x g for 2 min). Remove supernatant and immediately resuspend the pellet in 0.5–1 ml of RNA*later*. Yeast cells can be stored in RNA*later* for up to 8 hr at 25°C, or up to a week at 4°C.

For long-term storage, incubate the cells in RNA*later* for 1 hr. Repellet the cells (centrifuge at >12,000 x g for 5 min), remove supernatant, flash freeze, and store at –80°C.

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.

D. Storage in RNA*later*

Storage at –80°C

Storage at –80°C is recommended for archival samples and will provide optimal preservation. Samples can be stored at –80°C indefinitely. RNA*later* will freeze at –80°C.

To prepare samples for storage at –80°C, first incubate the samples overnight at 4°C to allow thorough penetration of the tissue, then transfer to –80°C. To expedite thawing of the samples, we recommend removing the tissue, or pelleting cells, from RNA*later* before freezing at –80°C.

Samples can subsequently be thawed at room temperature and refrozen without significantly affecting the amount or the integrity of the recoverable RNA.

Storage at –20°C

Storage at –20°C can also be used for archival samples. Samples will not freeze at –20°C, but crystals may form; this will not affect subsequent RNA isolation. Samples can be stored at –20°C indefinitely.

To prepare samples for storage at –20°C, first incubate the samples overnight at 4°C to allow thorough penetration of the tissue, then transfer to –20°C.

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

Most samples can be stored at 4°C for up to 1 month without significant RNA degradation.

If refrigeration is not available:

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

Storage at 25°C (room temp)

Most samples can be stored at 25°C in RNA*later* for up to 1 week without significant loss of RNA quality. After 2 weeks at 25°C RNA generally appears slightly degraded (marginally acceptable for Northern analysis, but still of sufficient quality for nuclease protection assays 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.

E. RNA Isolation from Samples in RNA*later*

1. Remove RNA*later* from samples

IMPORTANT

RNase inactivation is reversible; do not rinse RNAlater from samples before using. Blot tissues with a wipe, or pellet cells to remove excess RNAlater.

Tissue

Remove tissues from RNA*later* with sterile forceps, and then submerge it in RNA isolation lysis solution. Homogenize tissue promptly after placing it in lysis/denaturation solution.

Cells

There are two options for isolating RNA from cells stored in RNA*later*: The preferred method is to remove RNA*later* from the cells prior to extraction. Alternatively, cells in RNA*later* can be used directly for RNA extraction. Because of the greater volume that the cells are in, this method generally requires additional lysis solution.

- ***Removal of*** RNA*later* ***prior to extraction***

  Because of the density of RNA*later*, greater centrifugal forces are required to pellet cells from RNA*later* than normal media. Generally, cells become much less fragile when stored in RNA*later* and can be centrifuged at high speed without lysis. Most cell types can be centrifuged at 5000 x g without damage to the cells. Since different cell types vary in their ability to withstand centrifugal forces, we recommend testing the centrifugal speed with an expend-

able sample. Alternatively, dilute the RNA*later* by adding an equal volume of ice cold PBS (or other buffered solution) immediately before centrifugation to reduce the density of the solution, then centrifuge at normal speeds.

- ***RNA extraction from cells in RNAlater***

  One-step phenol-based disruption/extraction solutions, such as Ambion's TRI Reagent® or RNAwiz™ (available only in Japan), can be used to purify RNA from cells suspended in RNA*later*. This can be done by adding ten volumes of the one-step solution to the cell mixture, and proceeding normally. When 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

Lysates from RNA*later*-treated samples often require more force to pass through glass-fiber filters than lysates from untreated samples. Therefore, it may be necessary to use centrifugation instead of vacuum pressure to pass lysates through glass-fiber filters.

One-step disruption/extraction solutions

When using one-step RNA isolation products such as TRI Reagent or RNAwiz on RNA*later*-preserved samples, the aqueous phase will occasionally appear cloudy; this will not adversely affect RNA recovery or quality.

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.

F. RNA*later* Specifications

Storage and Stability

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

Quality Assurance:

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

Material Safety Data Sheets:

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from our website by going to the following address and clicking on the link for RNA*later*:
  www.ambion.com/techlib/msds
- Alternatively, email us at MSDS@ambion.com to request MSDSs by e-mail, fax, or ground mail. Specify the Ambion catalog number of the kit(s) for which you want MSDSs and whether you want to receive the information by e-mail, fax, or ground mail. Be sure to include your fax number or mailing address as appropriate. If the mode of receipt is not specified, we will e-mail the MSDSs.
- Customers without internet access can contact our technical service department by telephone, fax, or ground mail to request MSDSs (see contact information on the back of this booklet).

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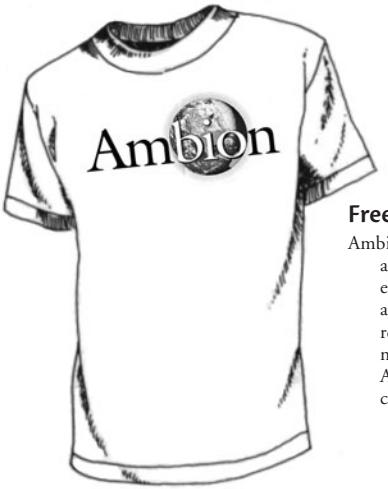

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Manual Version 0605

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