RNA*later*® Tissue Collection: RNA Stabilization Solution

Part Number AM7020 (100 mL), AM7024 (250 mL), AM7021 (500 mL), AM7022 (50 x 1.5 mL), AM7023 (20 x 5 mL)

Applied Biosystems

A. Product Description

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

Samples in RNA*later* Solution 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* Solution at –20°C or below.

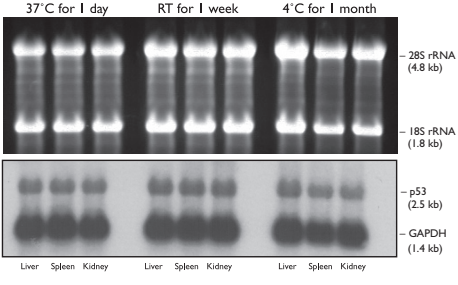

Figure 1. RNA from Tissue Stored in RNA*later*® Solution.

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

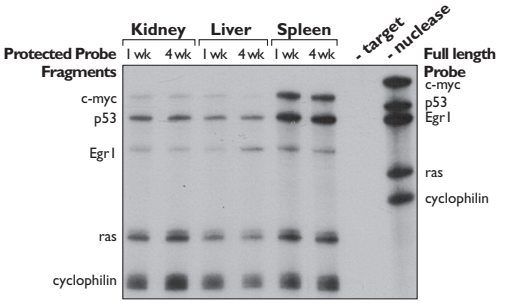

Figure 2. mRNA Profiles of Mouse Tissues Stored in RNA*later*® Solution.

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

B. Product Guidelines

Storage and stability

- Store RNA*later* Solution at room temperature.
- If any precipitation of RNA*later* Solution is seen, heat it to 37°C and agitate to redissolve it.

Disposal of RNA*later* Solution

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

Chemical reactivity with oxidants

WARNING

RNAlater Solution is known to react with hypochlorite solutions, such as common bleach. The reaction releases toxic chlorine gas, and is violent enough to generate heat. Similar reactions are expected from other oxidizing agents.

If you suspect that samples may contain bleach, work in a fume hood with adequate protective clothing and equipment.

Sample types compatible with RNA*later* Solution

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

RNA*later* Solution has been extensively tested with animal tissues, including brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung, and thymus. It 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 our citation database:

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

RNA isolation from RNA*later* Solution

RNA*later* Solution is compatible with most RNA isolation methods. Samples stored in RNA*later* Solution have been used successfully with TRI Reagent® (P/N AM9738), and all of Ambion® 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.

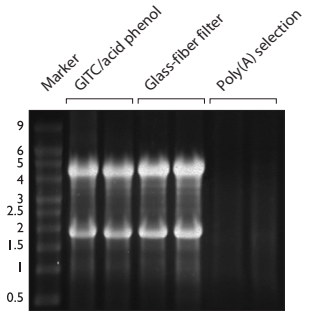

Figure 3. RNA isolated from tissue stored in RNA*later* Solution using different methods.

Whole mouse hearts (left lane of each set) and livers (right lane of each set) were dissected, and stored in RNA*later* Solution 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* Solution-stored samples

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

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

Isolating protein from RNA*later* Solution-stored samples

Proteins are also preserved in RNA*later* Solution. RNA*later* Solution will denature proteins; therefore, protein obtained from samples stored in it 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* Solution

- Use RNA*later* Solution with ***fresh tissue only***; do not freeze tissues before immersion in RNA*later* Solution.
- Before immersion in RNA*later* Solution, cut large tissue samples to ≤0.5 cm in any single dimension.
- Place the fresh tissue in 5–10 volumes of RNA*later* Solution.
- Most samples in RNA*later* Solution 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* Solution immediately; store at 4°C overnight (to allow the solution to thoroughly penetrate the tissue), remove supernatant, then move to –20°C or –80°C for long-term storage.

NOTE

Ambion offers RNAlater®-ICE (P/N AM7030) 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* Solution does not disrupt the structure of tissues; thus, tissue that has been equilibrated in RNA*later* Solution can be removed from the solution, sectioned into smaller pieces, and returned to RNA*later* Solution, if desired.

Small organs such as mouse liver, kidney and spleen can be stored whole in RNA*later* Solution.

Plant Tissue

Plant tissues that have natural barriers to diffusion, such as waxy coatings on leaves, will often require disruption to allow RNA*later* Solution access to the tissue. However, many plant tissues can simply be submerged in RNA*later* Solution 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* Solution. The cells can be washed in PBS before resuspending in RNA*later* Solution, if desired.

Blood and Plasma

White blood cells can be effectively preserved in RNA*later* Solution when separated from the red blood cells and sera and treated as tissue culture cells. RNA*later* Solution can also be added to small volumes of anticoagulated whole blood, sera, and plasma; however, the procedure is not presented here—see the Ambion RiboPure™ Blood Kit (P/N AM1928) protocol for detailed instructions.

Yeast

Pellet up to 3×10^8 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* Solution. Yeast cells can be stored in RNA*later* Solution 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* Solution 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* Solution is bacteriostatic; although bacteria do not grow in it, the cells remain intact. *E. coli* stored in RNA*later* Solution for 1 month at 4°C are intact and yield undegraded RNA.

D. Storage in RNA*later* Solution

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* Solution will freeze at –80°C.

To prepare samples for storage at –80°C, first incubate the samples in RNA*later* Solution 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 the RNA*later* Solution 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 in RNA*later* Solution 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 in RNA*later* Solution 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* Solution 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* Solution 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 3 days.

E. RNA Isolation from Samples in RNA*later* Solution

1. Remove RNA*later* Solution from samples

IMPORTANT

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

Tissue

Retrieve tissue from RNA*later* Solution with sterile forceps, quickly blot away excess RNA*later* Solution with an absorbent lab wipe or paper towel, and then submerge the sample 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* Solution: The preferred method is to remove the solution from the cells prior to extraction. Alternatively, cells in RNA*later* Solution 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** RNAlater Solution **prior to extraction***
  Because of the density of RNA*later* Solution, greater centrifugal forces are required to pellet cells from RNA*later* Solution than from normal media. Generally, cells become much less fragile when stored in RNA*later* Solution 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 expendable sample. Alternatively, dilute the RNA*later* Solution 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 Solution***
  One-step phenol-based disruption/extraction solutions, such as Ambion TRI Reagent® Solution or RNAWIZ™ Reagent (available only in Japan), can be used to purify RNA from cells suspended in RNA*later* Solution. 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* Solution-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 Solution or RNAWIZ Reagent (available only in Japan), on RNA*later* Solution-preserved samples, the aqueous phase will occasionally appear cloudy; this will not adversely affect RNA recovery or quality.

With RNAWIZ, there may be a problem getting the aqueous phase to mix with isopropanol at the precipitation step because of RNA*later* Solution 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* Solution was carried over; if the sample was mostly RNA*later* Solution, as much as an equal volume may be needed.

F. RNA*later*® Solution Specifications

P/N	Product Size
AM7020	100 mL
AM7021	500 mL
AM7024	250 mL
AM7022	50 x 1.5 mL
AM7023	20 x 5 mL

Storage and Stability

Store RNA*later* Solution at room temperature.

Quality Assurance:

RNA*later* Solution 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 product-specific links on our website at the following address:
  www.ambion.com/techlib/msds
- Alternatively, e-mail your request to:
  MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
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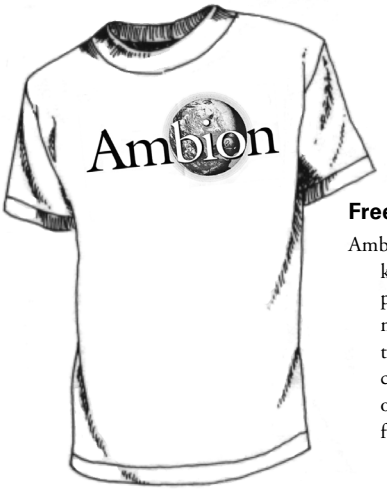

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