

## Product Information

### RNA<sup>later</sup>®

#### RNA Stabilization Solution for Tissue

Catalog Number **R0901**

Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

RNA<sup>later</sup>® 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<sup>later</sup> for storage without jeopardizing the quality or quantity of RNA. RNA<sup>later</sup> eliminates the need to immediately process tissue specimens or to freeze samples in liquid nitrogen for later processing.

RNA<sup>later</sup> preserves RNA in tissues for up to 1 day at 37 °C, 1 week at 25 °C, and 1 month at 4 °C. Tissues can also be stored at –20 °C long-term.

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

RNA<sup>later</sup> is compatible with most RNA isolation methods, including TRI Reagent® and GenElute™ total RNA isolation and mammalian mRNA isolation kits.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store RNA<sup>later</sup> at room temperature. If a precipitate develops in the RNA<sup>later</sup>, warm the solution to 37 °C and agitate to redissolve it.

### Procedure

Use RNA<sup>later</sup> with fresh tissue only. **Do not freeze tissue** before immersion in RNA<sup>later</sup>.

#### A. Preparation of Tissue Samples

##### 1. Animal Tissue

Cut tissue samples to a maximum thickness in any one dimension of 0.5 cm (e.g., 0.5 cm × 1 cm × 1 cm), place the fresh tissue in 5 volumes of RNA<sup>later</sup>, and store as indicated for the desired temperature in section B.

RNA<sup>later</sup> does not dissolve or disrupt the structure of tissue samples. Small organs such as rat liver, kidney, and spleen can be stored whole in RNA<sup>later</sup>.

##### 2. Plant Tissue

Many plant tissues can be simply submerged in 5 volumes of RNA<sup>later</sup> for storage. RNA<sup>later</sup> has been tested for isolation of 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 require disruption to allow RNA<sup>later</sup> access to the tissue. Any method of disruption that breaks up the waxy coating (e.g., dicing or physically tearing) is suitable.

##### 3. Tissue Culture Cells

Pellet cells according to standard laboratory protocols. Wash the cells with PBS or an equivalent buffer to remove the culture medium. Resuspend the cells in a small volume of PBS to "loosen up" the cell pellet so the RNA<sup>later</sup> can penetrate the cells more easily. After resuspending the cells, add 5–10 equivalent volumes of RNA<sup>later</sup> to the cell suspension. No further rinsing of the cell pellet is necessary.

4. White Blood Cells  
White blood cells can be effectively preserved in *RNA/later* if they are separated from the red blood cells and serum, and treated as tissue culture cells.
  5. Anticoagulated Blood  
Gently invert collection tube several times to mix blood sample. Add 300-500 $\mu$ L of anticoagulated blood to 1.3 mL of *RNA/later*. Mix thoroughly. Sample can be stored in ambient for up to 3 days and at -20 $^{\circ}$ C for long term storage.
  6. Bacteria  
*RNA/later* is bacteriostatic; although bacteria do not grow in *RNA/later*, the cells remain intact. *E. coli* cells stored in *RNA/later* for 1 month at 2-8 $^{\circ}$ C are intact and yield undegraded RNA.
- B. Storage of Samples in *RNA/later*
1. Storage at -80  $^{\circ}$ C (recommended for long-term storage)  
Incubate samples at 2-8 $^{\circ}$ C overnight, then remove them from *RNA/later* before storage at -80  $^{\circ}$ C to prevent the formation of salt crystals. For tissue culture cells, do not remove the *RNA/later*, simply freeze the whole solution. The cell types that have been evaluated do not lyse when frozen at -80  $^{\circ}$ 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.
  2. Storage at -20  $^{\circ}$ C (also an option for long-term storage)  
Incubate samples at 2-8 $^{\circ}$ C overnight, then transfer to -20  $^{\circ}$ C. Samples will not freeze at -20  $^{\circ}$ C, but crystals may form in the storage buffer; this will not affect subsequent RNA isolation. If crystals are a concern, remove the *RNA/later* prior to storing the samples at -20  $^{\circ}$ C. Samples can subsequently be thawed at room temperature and refrozen without affecting the amount or the integrity of the recoverable RNA.
  3. Storage at 2-8 $^{\circ}$ C  
Samples can be stored at 2-8 $^{\circ}$ C for up to 1 month without any experimental evidence of RNA degradation.
  4. Storage at ambient temperature (25  $^{\circ}$ C)  
RNA isolated from samples stored at 25  $^{\circ}$ C for one week is intact. RNA from samples stored at 25  $^{\circ}$ C for two weeks appears slightly degraded (i.e., marginally acceptable for Northern analysis, but still of sufficient quality for nuclease protection assay or RT-PCR analysis). If ambient temperature is above 25  $^{\circ}$ C, incubate samples in *RNA/later* on ice for a few hours if possible before storing at ambient temperature.
  5. Storage at 37  $^{\circ}$ C  
RNA isolated from samples stored at 37  $^{\circ}$ C is intact after a 24 hour incubation, but is partially degraded after a 3 day incubation.
- C. RNA Isolation from Material in *RNA/later*  
**Note:** If 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.
1. 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.  
**Note:** Tissues stored in *RNA/later* develop a hard, rubbery texture, and may be more difficult to homogenize thoroughly than fresh tissue. Dicing the tissue into smaller pieces with a scalpel can expedite homogenization. Animal tissue that has been stored in *RNA/later* can be removed from the solution, sectioned into smaller pieces, and returned to *RNA/later* if desired.
  2. Cells stored in *RNA/later* can either be removed by centrifugation or the RNA can be extracted from the mixture of cells and *RNA/later*.

3. Centrifugation - Since *RNAlater* is more dense than typical cell culture media, the cells may not pellet at the centrifugal force normally used for live cells. Pellet cells by centrifugation and remove the *RNAlater* by aspiration. For HeLa cells, centrifugation at  $\sim 3,000 \times g$  is suitable, but other cell types may not tolerate this speed or may require more force to pellet.
4. Disruption/Extraction - RNA may be extracted from cells stored in *RNAlater* using a one-step disruption/extraction solution, e.g., TRI Reagent, Catalog Number T9424. This can be done by adding ten volumes of the one-step solution to the cell mixture and proceeding normally. When using other disruption/extraction solutions, it may be necessary to dilute the aqueous phase before the RNA precipitation step.  
**Note:** When using one-step RNA isolation products such as TRI Reagent on *RNAlater*-preserved samples, the aqueous phase may be cloudy. If this occurs, simply continue the procedure, following the technical bulletin or manufacturer's instructions. Cloudiness of the aqueous phase does not affect the quantity or quality of the RNA obtained.

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