



Product Information

RNAlater™ **RNA Stabilization Solution for Tissue**

Product Code **R 0901**
Store at room temperature

Product Description

RNAlater™ 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 RNAlater for storage without jeopardizing the quality or quantity of RNA. RNAlater eliminates the need to immediately process tissue specimens or to freeze samples in liquid nitrogen for later processing.

RNAlater 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.

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

RNAlater is compatible with most RNA isolation methods, including TRI Reagent®* and GenElute total RNA isolation and mammalian mRNA isolation kits.

Disclaimer

RNAlater is for R&D use only. Not for drug, household or other uses.

Storage and Stability

Store RNAlater at room temperature. If a precipitate develops in the RNAlater, warm the solution to 37 °C and agitate to redissolve it.

Procedure

Use RNAlater with fresh tissue only. **Do not freeze tissue** before immersion in RNAlater.

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 x 1 cm x 1 cm), place the fresh tissue in 5 volumes of RNAlater, and store as indicated for the desired temperature in part B below. RNAlater does not dissolve or disrupt the structure of tissue samples. Small organs such as rat liver, kidney and spleen can be stored in RNAlater whole.
2. **Plant Tissue**
Many plant tissues can be simply submerged in 5 volumes of RNAlater for storage. RNAlater 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 RNAlater 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 that the *RNA/later* can penetrate the cells more easily. After resuspending the cells, add 5 to 10 equivalent volumes of *RNA/later* 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. *RNA/later* is not recommended for preserving RNA in whole blood, plasma, or serum. Because of their high protein content, these fluids will form an insoluble precipitate if they are mixed with *RNA/later*.
5. **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.

B. Storage of Samples in *RNA/later*

1. **Storage at -80 °C**
Storage at -80 °C is recommended for long-term storage. Incubate samples at 4 °C overnight, then remove them from *RNA/later* before storage at -80 °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 °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 °C**
Storage at -20 °C is recommended for long-term 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. If crystals are a concern, remove the *RNA/later* prior to storing the samples at -20 °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 4 °C**
Samples can be stored at 4 °C for up to 1 month without any experimental evidence of RNA degradation.
4. **Storage at ambient temperature**
RNA isolated from samples stored at 25 °C for one week is intact. RNA from samples stored at 25 °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 °C, incubate samples in *RNA/later* on ice for a few hours if possible before storing at ambient temperature.
5. **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.

C. RNA Isolation from Material in *RNA/ater*

After removing *RNA/ater* from samples, it can be discarded down the sink with running water.

1. Tissues that have been stored in *RNA/ater* 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 that tissues stored in *RNA/ater* develop a hard, rubbery texture, and may be more difficult to homogenize thoroughly than would fresh tissue. Dicing the tissue into smaller pieces with a scalpel can expedite homogenization. Animal tissue that has been stored in *RNA/ater* can be removed from the solution, sectioned into smaller pieces, and returned to *RNA/ater* if desired.
2. Cells stored in *RNA/ater* can either be removed by centrifugation, or the RNA can be extracted from the mixture of cells and *RNA/ater*.
3. Centrifugation: Since *RNA/ater* 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 *RNA/ater* by aspiration. ~3000 x g is suitable for HeLa cells, 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 *RNA/ater* using a one-step disruption/extraction solution (e.g. TRI Reagent, Product Code 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.

Additional Notes for RNA Isolation

1. 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.
2. When using one-step RNA isolation products such as TRI Reagent on *RNA/ater*-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.

RNA/ater is a trademark of Ambion Inc., Austin, Texas, and is covered by various U.S. and foreign patents. TRI Reagent is a registered trademark of Molecular Research Center Inc.

Related Products

GenElute™ Mammalian Total RNA Miniprep Kits, Product Code RTN-10, RTN-70, RTN-350
 GenElute™ Direct mRNA Miniprep Kits, Product Code DMN-10, DMN-70
 GenElute™ mRNA Miniprep Kits, Product Code MRN-10, MRN-70
 TRI Reagent, Product Code T9424

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