



## Product Information

### RNAlater Reagent

Catalog Number	Packaging Size
R140	100 mL

#### Storage upon receipt:

- RT

### Product Description

RNAlater Reagent 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 RNAlater Reagent and stored for analysis at a later date.

Samples in RNAlater Reagent can be stored for extended periods under conditions where RNA degradation would normally take place rapidly. Tissues can be stored indefinitely in RNAlater Reagent at  $-20^{\circ}\text{C}$  or below.

### Sample types compatible with RNAlater Reagent

RNAlater Reagent 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.

RNAlater Reagent 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.

### Compatible RNA isolation methods

RNAlater Reagent is compatible with one-step RNA isolation methods as well as methods that use silica membrane binding, acid phenol extraction, or oligo(dT) selection of mRNA.

### Guidelines for use of RNAlater Reagent

- ❖ Use RNAlater Reagent with fresh tissue only; do not freeze tissues before immersion in RNAlater Reagent.
- ❖ Before immersion in RNAlater Reagent, cut large tissue samples to  $\leq 0.5$  cm in any single dimension.
- ❖ Place the fresh tissue in 5-10 volumes of RNAlater Reagent.
- ❖ Most samples in RNAlater Reagent can be stored at room temperature for 1 week without compromising RNA quality or at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  indefinitely.
- ❖ Do not freeze samples in RNAlater Reagent immediately; store at  $4^{\circ}\text{C}$  overnight (to allow the solution

to thoroughly penetrate the tissue), remove supernatant, then move to  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  for long-term storage.

### Animal tissue

RNAlater Reagent does not disrupt the structure of tissues; thus, tissue that has been equilibrated in RNAlater Reagent can be removed from the solution, sectioned into smaller pieces, and returned to RNAlater Reagent, if desired.

Small organs such as mouse liver, kidney and spleen can be stored whole in RNAlater Reagent.

### Plant tissue

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

### Blood and plasma

White blood cells can be effectively preserved in RNAlater Reagent when separated from the red blood cells and sera and treated as tissue culture cells.

### Yeast

Pellet up to  $3 \times 10^8$  cells (centrifuge at  $12,000 \times g$  for 2 min). Remove supernatant and immediately resuspend the pellet in 0.5–1 mL of RNAlater Reagent. Yeast cells can be stored in RNAlater Reagent for up to 8 hr at  $25^{\circ}\text{C}$ , or up to a week at  $4^{\circ}\text{C}$ . For long-term storage, incubate the cells in RNAlater Reagent for 1 hr. Repellet the cells (centrifuge at  $>12,000 \times g$  for 5 min), remove supernatant, flash freeze, and store at  $-80^{\circ}\text{C}$ .

### Bacteria

RNAlater Reagent is bacteriostatic; although bacteria do not grow in it, the cells remain intact. *E. coli* stored in RNAlater Reagent for 1 month at  $4^{\circ}\text{C}$  are intact and yield undegraded RNA.

### Storage in RNAlater Reagent

#### Storage at $-80^{\circ}\text{C}$

Storage at  $-80^{\circ}\text{C}$  is recommended for archival samples and will provide optimal preservation. Samples can be stored at  $-80^{\circ}\text{C}$  indefinitely. RNAlater Reagent will freeze at  $-80^{\circ}\text{C}$ .

To prepare samples for storage at  $-80^{\circ}\text{C}$ , first incubate the samples in RNAlater Reagent overnight at  $4^{\circ}\text{C}$  to allow thorough penetration of the tissue, then transfer to  $-80^{\circ}\text{C}$ . To expedite thawing of the samples, we recommend removing the tissue, or pelleting cells, from the RNAlater Reagent before freezing at  $-80^{\circ}\text{C}$ .

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 RNAlater Reagent 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 RNAlater Reagent at 4°C for up to 1 month without significant RNA degradation.

### **Storage at 25°C**

Most samples can be stored at 25°C in RNAlater Reagent for up to 1 week without significant loss of RNA quality. After 2 weeks at 25°C, RNA generally appears slightly degraded.

### **Storage at 37°C**

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

## **RNA isolation from samples in RNAlater Reagent**

### **Tissue**

Retrieve tissue from RNAlater Reagent with sterile forceps, quickly blot away excess RNAlater Reagent 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**

Because of the density of RNAlater Reagent, greater centrifugal forces are required to pellet cells from RNAlater Reagent than from normal media. Generally, cells become much less fragile when stored in RNAlater Reagent 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 RNAlater Reagent 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.