

★ Storage

Store at RT

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★ Introduction

RNA Guardian 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 Guardian Solution and stored for analysis at a later date. Samples in RNA Guardian Solution can be stored for extended periods under conditions where RNA degradation would normally take place rapidly. Tissues can be stored indefinitely in RNA Guardian Solution at -20°C or below.

★ Important Product Notice**Storage and stability**

- Store RNA Guardian Solution at room temperature.
- If any precipitation of RNA Guardian Solution is seen, heat it to 37°C and agitate to redissolve it.
- If the crystals do not go into the solution at 37°C, loosen the cap and heat the solution at a higher temperature, up to 65°C, for ~30 minutes, mixing periodically. Once the crystals have dissolved, store the solution in smaller aliquots in case crystals re-form after cooling. Warming the solution in this way does not affect performance of RNA Guardian Solution.

Sample types compatible with

RNA Guardian 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 Guardian 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.

Compatible RNA isolation methods

RNA Guardian Solution is compatible with one-step RNA isolation methods as well as methods that use glass binding, acid phenol extraction, or oligo(dT) selection of mRNA.

★ Important Guidelines for Use of RNA Guardian Solution**General Guidelines**

- Use RNA Guardian Solution with fresh tissue only; do not freeze tissues before immersion in RNA Guardian Solution.
- Before immersion in RNA Guardian Solution, cut large tissue samples to ≤ 0.5 cm in any single dimension.
- Place the fresh tissue in 5–10 volumes of RNA Guardian Solution.
- Most samples in RNA Guardian Solution can be stored at room temperature for 1 week without compromising RNA quality, or at -20°C indefinitely.
- Do not freeze samples in RNA Guardian 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.

Animal tissue

RNA Guardian Solution does not disrupt the structure of tissues; thus, tissue that has been equilibrated in RNA Guardian Solution can be removed from the solution, sectioned into smaller pieces, and returned to RNA Guardian Solution, if desired. Small organs such as mouse liver, kidney and spleen can be stored whole in RNA Guardian Solution

Plant tissue

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

Blood and plasma

White blood cells can be effectively preserved in RNA Guardian Solution when separated from the red blood cells and sera and treated as tissue culture cells. RNA Guardian Solution can also be added to small volumes of anticoagulated whole blood, sera, and plasma; however, the procedure is not presented here.

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 Guardian Solution. Yeast cells can be stored in RNA Guardian 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 Guardian 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 Guardian Solution is bacteriostatic; although bacteria do not grow in it, the cells remain intact. *E. coli* samples stored in RNA Guardian Solution for 1 month at 4°C are intact and yield undegraded RNA.

★ Sample Storage in RNA Guardian 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 Guardian Solution will freeze at -80°C. To prepare samples for storage at -80°C, first incubate the samples in RNA Guardian 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 Guardian 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 Guardian 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 Guardian Solution at 4°C for up to 1 month without significant RNA degradation. Place samples in the coolest environment available. If the room ambient temperature is above 25°C, incubate the samples in RNA Guardian Solution on ice for a few hours, if possible, before storing at ambient temperature.

Storage at 25°C (room temperature)

Most samples can be stored at 25°C in RNA Guardian 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-hours incubation, but is partially degraded after 3 days.

★ Remove RNA Guardian Solution from Samples for RNA Isolation

Tissue

Retrieve tissue from RNA Guardian Solution with sterile forceps, quickly blot away excess RNA Guardian 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.

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

Cells

There are two options for isolating RNA from cells stored in RNA Guardian Solution. The preferred method is to remove the solution from the cells prior to extraction. Alternatively, cells in RNA Guardian 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 RNA Guardian Solution prior to extraction

Because of the density of RNA Guardian Solution, greater centrifugal forces are required to pellet cells from RNA Guardian Solution than from normal media. Generally, cells become much less fragile when stored in RNA Guardian Solution and can be centrifuged at high speed without lysis. Most cell types can be centrifuged at 5,000 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 Guardian 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 RNA Guardian Solution

One-step phenol-based disruption/extraction solutions, such as PureExtract RNAsol can be used to purify RNA from cells suspended in RNA Guardian Solution. This can be done by adding ten volumes of the one-step solution to the cell mixture and proceeding normally. When RNAwiz Reagent is used in this way, it may be necessary to dilute the aqueous phase before the RNA precipitation step.

Note: Lysates from RNA Guardian 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.

Note: When using one-step RNA isolation products such as PureExtract RNAsol on RNA Guardian Solution-preserved samples, the aqueous phase will occasionally appear cloudy; this will not adversely affect RNA recovery or quality.

★ Safety Information

To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions.