MB469_IFU_EN_V2301

RNA Stabilization Solution

Catalogue number	Presentation
MB46901	100 mL
MB46902	500 mL

Description

Reliable gene expression analysis hinges on the immediate stabilization of RNA in biological samples upon harvesting. Gene expression patterns can change due to RNA degradation and transcriptional shifts, making it imperative to halt these changes for accurate results in quantitative gene expression analyses, such as microarray analysis and quantitative RT-PCR. RNA Stabilization Solution offers an advanced way to stabilize and protect RNA within intact tissue samples, eliminating the urgency to process or deep-freeze samples instantly. Once tissues are harvested, they can be directly immersed in the RNA Stabilization Solution, ensuring that the RNA remains intact even during storage or transport at varying temperatures. The solution is ideal for streamlining workflows, replacing complex and hazard-prone methods such as snap-freezing in liquid nitrogen or immediate sample processing. Tissue samples, once treated, can be preserved in the RNA Stabilization Solution for up to 1 day at 37°C, 1 week at 25°C and 1 month at 4°C. For long-term storage, tissues can be maintained indefinitely at -20°C or colder (-80°C). The solution's versatility is underscored by its effectiveness across various tissues from vertebrate species, bacterial cultures, tissue culture cells, white blood cells, and even some plants.

RNA Stabilization Solution is compatible with a broad spectrum of RNA isolation methods, including but not limited to TRIzol/NZYol Reagent (Cat. No. MB18501) and standard RNA isolation kits. Moreover, it has been used in downstream applications ranging from mRNA and total RNA isolation to histology, immunocytochemistry, cDNA library construction, Northern blotting, RNA sequencing (RNA-seq), RT-PCR and RT-qPCR, RNA interference (RNAi) studies, MicroRNA (miRNA) analysis and additional techniques. RNA Stabilization Solution is specifically crafted for molecular biology applications. This product is not developed for the diagnosis, prevention, or treatment of any disease.

Sample compatibility and RNA Isolation methods

RNA Stabilization Solution is versatile and effective in preserving RNA from a broad range of biological sources. This includes most animal tissues (*e.g.* brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung, and thymus), cultured cells, bacteria and yeast. Specific samples, such as *E. coli*, Drosophila, tissue culture cells, white blood cells, and certain plant tissues, are also suitable for use. However, the effectiveness of RNA Stabilization Solution may be limited in samples that are resistant to penetration, such as waxy plant tissues and bone. The solution is compatible with a variety of RNA isolation techniques, from one-step RNA isolation methods to those leveraging glass binding, acid phenol extraction, or oligo(dT) mRNA selection. Samples stored in RNA Stabilization Solution can be used to extract both DNA and RNA, as DNA is preserved along with RNA. It is important to mention that RNA Stabilization Solution preserves proteins but denatures them. Consequently, proteins from these samples will be apt for applications like Western blotting or 2D gel electrophoresis, but not suitable for assays requiring native protein forms.

Key Advantages & Features

- Efficient RNA Preservation: Stabilizes RNA for 1 day at 37°C, 1 week at 25°C, 1 month at 4°C and at -20°C for long-term storage.
- Immediate RNase Inactivation: A single reagent that rapidly inactivates RNases, safeguarding RNA integrity within tissues and cells.
- Simplified Storage & Handling: Eliminates the need for immediate freezing in liquid nitrogen or storing in lab freezers.
- Suitable for field collection: Specially crafted for on-site tissue collection, perfect for locations like hospitals where immediate RNA processing is not feasible.
- Versatility in RNA Isolation: Compatible with a broad range of RNA isolation techniques, including the complete range of NZYtech RNA isolation kits.
- High-Quality RNA Extraction: Ensures uncompromised RNA quality, whether extracting mRNA or total RNA.
- Rapid Tissue Permeation: Quickly penetrates tissues to offer maximum RNA stabilization and protection.
- Safe & Non-toxic: An aqueous solution that poses no harm, allowing seamless downstream tissue processing.
- Reliable Preservation: Offers consistent tissue preservation without RNA degradation and is ideal for archiving tissues for future microdissection.
- Enhanced Protection: Particularly beneficial for tissues rich in RNases, ensuring RNA integrity.
- Flexibility in Tissue Storage: Samples can be stored at room temperature, allowing for convenient sample collection at different time points without immediate processing, or shipping samples on wet ice or even overnight at room temperature.
- Broad Application: Useful for diverse scenarios, from stabilizing RNA during lengthy dissections, and gathering samples in unique environments to facilitating gene expression and gene-profiling studies.

Protocol

RNA Stabilization Solution is designed to safeguard RNA in various samples, ensuring its stability and protection.

1. Sample Preparation:

Tissue Samples: Limit dimensions to ≤0.5 cm. This size restriction ensures efficient solution penetration. Excessively thick samples (>0.5 cm) may result in uneven solution penetration, risking RNA degradation.

Small Organs (*e.g.*, mouse kidney, liver, spleen): These can be entirely immersed in the RNA Stabilization Solution. Once ready for RNA isolation, remove the tissue and proceed as if freshly harvested.

Cells: Resuspend pelleted cells in a minimal amount of PBS. Then, introduce 5-10 volumes of RNA Stabilization Solution. Before RNA extraction, pellet the cells and discard the supernatant.

2. Procedure:

Submerge the cells, small organs, or dissected tissue (≤0.5 cm in any dimension) in approximately 5 volumes of RNA Stabilization Solution at room temperature, allowing the reagent to permeate and stabilize the RNA. Only use the solution with fresh tissue—do not freeze beforehand. While weighing the tissues provides accuracy, unstabilized RNA may degrade during the process. In some instances, it can be more practical to estimate the weight of tissue samples. Use the table below as a reference (in this case around 10 volumes of RNA Stabilization Solution are recommended as a precautionary measure; correct weight is not available):

Mouse organ	Average Weight (mg)	Minimum amount of RNA Stabilization Solution (mL)
Kidney	180–250	1.8–2.5
Spleen	100–160	1–1.6
Lung	190–210	1.9–2.1
Heart	100–170	1–1.7
Liver	1000–1800	10–18

Generally, 5 volumes of the RNA stabilization solution suffice. However, when estimating tissue weight or for larger samples, utilizing 10 volumes ensures optimal RNA preservation.

3. Special Considerations:

- Animal Tissues: The solution maintains tissue structure, making it ideal for storage of tissues like mouse liver, kidney, and spleen.
- Plant Tissues: While some may require disruption for solution access, many can be directly submerged.
- Tissue Culture Cells: After pelleting, introduce 5–10 volumes of RNA Stabilization Solution.
- Blood and Plasma: The solution preserves white blood cells when segregated from other elements.
- Yeast: After pelleting and resuspension in the solution, it's suitable for storage. For prolonged periods, consider flash freezing post-incubation.
- Bacteria: For instance, *E. coli* can be stored in this solution at 4°C for up to a month, yielding intact RNA.

Sample Storage Conditions Using RNA Stabilization Solution

1. Storage at -80°C:

- Purpose: Best for archival samples, offering optimal RNA preservation.
- Duration: Indefinitely.
- Procedure:
  - RNA Stabilization Solution will freeze at this temperature.
  - Pre-condition the samples in RNA Stabilization Solution overnight at 4°C to ensure comprehensive penetration.
  - Move the samples to -80°C for storage.
  - For faster thawing, remove tissue or pellet cells from the solution before freezing.
- Thawing & Refreezing: Samples can be thawed at room temperature and refrozen without compromising RNA amount or integrity.

2. Storage at -20°C:

- Purpose: Suitable for archival samples.
- Duration: Indefinitely.
- Procedure:
  - The solution will not freeze but may form crystals. This doesn't impact RNA isolation.
  - Like storage at –80°C, pre-condition the samples overnight at 4°C, then transfer to -20°C.
- Thawing & Refreezing: Samples can be thawed and refrozen at room temperature without harming the RNA's amount or integrity.

3. Storage at 4°C:

- Duration: Up to 1 month.
- Most samples when stored in the RNA Stabilization Solution at this temperature, will not experience significant RNA degradation.

4. Unavailability of Refrigeration:

- In the absence of cooling resources, keep samples in the coolest area available.
- If the surrounding temperature exceeds 25°C, incubate samples in RNA Stabilization Solution on ice for several hours (if feasible) before maintaining at ambient temperature.

5. Storage at 25°C (Room Temperature):

- Duration: Up to 1 week without significant RNA quality reduction.
- Note: After 2 weeks, the RNA may appear slightly degraded but remains adequate for various analyses, including Northern analysis, nuclease protection assays or RT-PCR.

6. Storage at 37°C:

- RNA remains intact after a 24-hour incubation. Partial degradation is observed after 3 days.

RNA isolation

Always consult NZYtech or other manufacturers' extraction kit handbooks before proceeding to RNA purification. Extraction RNA kit instructions offer vital instructions on selecting the correct starting material amount and determining the optimal method for tissue disruption and homogenization. For precise quantification, weigh the starting material. Tissues in RNA Stabilization Solution maintain their structural integrity. However, they might become slightly tougher than fresh tissues, although in this solution samples are still amenable to efficient disruption and homogenization. Tissues preserved at -30 to -15°C can be thawed and weighed at room temperature without affecting RNA integrity for up to 20 freeze–thaw cycles. Removal of RNA Stabilization Solution needs to be performed with care since RNase inactivation is reversible. Thus, do not rinse off the RNA Stabilization Solution. For tissues, use sterile forceps to retrieve, and blot away the excess solution using a lab wipe, then promptly immerse the tissue in the RNA isolation lysis solution to initiate homogenization. For cells, two methods are recommended:

- Prior Solution Removal: Cells become robust in the RNA Stabilization Solution, allowing for high-speed centrifugation (up to 5000 x g) without damage. If uncertain about the centrifugation speed, test with a sample or dilute the solution with an equal volume of ice-cold PBS before centrifuging at conventional speeds.
- Direct RNA Extraction: Use a one-step phenol-based disruption/extraction solution. Add 10 volumes of the one-step solution to the cell mixture and continue the process. When using NZYol Reagent (Cat. No. MB185), consider diluting the aqueous phase before the RNA precipitation step.

Shipping Conditions

This product is shipped at room temperature.

Storage Conditions

Store at room temperature, *i.e.* 15ºC to 25ºC; the solution remains stable for at least 12 months under these conditions. Freezing is not recommended.

If precipitation is observed:

- Initially, warm to 37°C and agitate to redissolve.
- If unsuccessful, slightly loosen the cap and heat up to 65°C for approximately 30 minutes, mixing occasionally.
- After dissolving any crystals, consider storing the solution in smaller aliquots to prevent re-crystallization.

  Note: Warming does not compromise the solution's efficacy.