

# RNASafer<sup>®</sup> Stabilizer Reagent

**WARNING:** This reagent is toxic if swallowed. After contact with skin, wash immediately with copious amounts of mild detergent and water.

Product No.: R0424-01 (50 ml), R0424-02 (250ml)

**Storage Conditions:** RNASafer<sup>®</sup> is stable for at least 12 months when stored at 15°C-20°C and yields reproducible results.

## Introduction

One of the major difficulties for RNA research is the RNA degradation during collection, storage and transportation of samples. It is extremely important to immediately stabilize RNA in biological samples because of changes in the gene-expression patterns occur due to specific and nonspecific RNA degradation. Avoiding such gene-expression pattern is essential for all reliable quantitative gene-expression analysis such as biochip and array analysis, and quantitative RT-PCR.

RNASafer<sup>®</sup> Reagent is a one reagent system for the preservation of total RNA from animal tissues and other biological samples. The reagent can protect RNA during transportation and storage at ambient temperatures. Large numbers of samples can be easily processed without the need of freezing with liquid nitrogen or dry ice. Once the sample is submerged in this RNASafer<sup>®</sup> Reagent, the RNASafer<sup>®</sup> rapidly permeates tissue and single cells to stabilize and protect cellular RNA. RNA samples stored in RNASafer<sup>®</sup> can last up to 24 hours at 30°C-37°C, 7 days at room temperature (20°C-25°C), 12 months at -20°C. RNASafer<sup>®</sup> reagent provide an alternate method to replace current inconvenient, dangerous and equipment intensive methods such as snap storage in liquid nitrogen or store at -80°C..

This reagent is suitable for small quantities of animal tissues (<150 mg) cell culture cells, and white blood cells. The simplicity of the RNASafer<sup>®</sup> Reagent method allows simultaneous processing of a large number of samples.

## General Notes Regarding Starting Material:

### A. Handling starting material

Since the RNA in tissues do not have any protection until the samples are treated with RNASafer<sup>®</sup> Reagent, it is extremely important to treat the sample with RNASafer<sup>®</sup> Reagent immediately after harvesting the material.

### B. Maximum tissue size

RNASafer<sup>®</sup> Reagent penetrates the sample by diffusion to protect cellular RNA. The reagent diffuses into the cells or into surface layer of solid tissues immediately after it contact the samples. So the samples size is critical for the successful result. **The ideal samples slices should be less than 0.25 cm thick.**

### C. Estimation of volume of RNASafer<sup>®</sup> Reagent to be applied

In order to protect RNA, the surface of the tissue samples should be completely covered by RNASafer<sup>®</sup> Reagent. It is strongly recommended that the sample should be put into at **least 15 volumes of RNASafer<sup>®</sup> Reagent.**

## Precaution

**Make sure that samples remain submerged at any time during storage and transportation.**

## Process samples with RNASafer<sup>®</sup> Reagent

### A. Culture cells, white blood cells

Pellet the cells by centrifugation at 300 x g for 10 min, Discard supernatant and wash cells with PBS. Resuspend the cells with a small volume of PBS (50-100µl) and add appropriate volume (500 µl to 1ml) of RNASafer<sup>®</sup> Reagent.

### B. Solid tissue samples

1. Harvest samples.
2. Cut the samples into smallest possible slices (< **0.25mm**)
3. Immediately drop the sample into a container with appropriate volume of RNASafer<sup>®</sup> Reagent. Make sure that the sample is fully covered by the reagent.

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## RNASafer<sup>®</sup> Protocol for Total RNA Isolation

**CAUTION:** When working with RNASafer<sup>®</sup> Reagent use gloves and eye protection (safety goggles) and avoid contact with skin or clothing. Unless otherwise noted, all steps are to be carried out at room temperature (20°C-25°C).

### A. Protocol for total RNA purification from tissue sample with E.Z.N.A.<sup>®</sup> Total RNA System

1. Remove sample from RNASafer<sup>®</sup> Reagent using forceps.
2. Estimate the amount of the tissue and select appropriate E.Z.N.A.<sup>®</sup> Total RNA Kit. Use RNA Miniprep Kit (R6634) for less than 40mg tissue, Total RNA Midiprep kit (R6664) for 200mg tissue, RNA Maxiprep Kit(R6693) for 1 gram tissue.
3. For RNA isolation using RNA miniprep kit, add 700µl TRK lysis buffer. Homogenize the tissue with a rotor-stator homogenizer. For RNA isolation using Midi and Maxiprep kit, follow the standard protocol in the user manual.
4. Follow standard protocol for E.Z.N.A.<sup>®</sup> Total RNA Kits in user manuals.

### B. Protocol for total RNA purification from culture cells and white blood cells with E.Z.N.A.<sup>®</sup> Total RNA System

1. For RNA isolation using RNA miniprep kit, take 150ul cell/RNASafer<sup>®</sup> Reagent mixture to a RNase free 1.5 ml tube. **Add 200ul TRK lysis buffer and mix with 350ul 70% ethanol.** For RNA isolation using Midi and Maxiprep kit, follow the standard protocol in the user manual.
2. Apply the sample to the HiBind<sup>®</sup> RNA column and follow standard protocol in user manual for each kits .

*For laboratory research use only.*

**CAUTION:** Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

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