


E.Z.N.A.[®] RNASafer™ II Reagent


R0424-00	5 mL
R0424-01	50 mL
R0424-02	250 mL


Manual Date: November 2021
Revision Number: v1.0

For Research Use Only

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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. Stabilization is essential for all reliable quantitative gene-expression analysis such as biochip and array analysis, and quantitative RT-PCR.

RNA Safer™ II Reagent is a single 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. A large number of samples can be easily processed without the need of freezing with liquid nitrogen or dry ice. Once the sample is submerged in RNA Safer™ II Reagent, the reagent rapidly permeates tissue and cells to stabilize and protect total cellular RNA. Samples stored in RNA Safer™ II Reagent can last up to 24 hours at 30-37°C, 7 days at room temperature (20-25°C), or 12 months at -20°C. RNA Safer™ II Reagent provides an alternative method to the current inconvenient, dangerous, and equipment intensive methods such as storage in liquid nitrogen or -80°C freezer.

RNA Safer™ II Reagent is suitable for most animal tissues, cultured cells, and white blood cells. The simplicity of the RNA Safer™ II Reagent method allows simultaneous processing of a large number of samples.

Stabilized samples could be applied to most RNA isolation methods, such as TRIzol, RNeasy and E.Z.N.A.® Total RNA Kit. Samples in the RNA Safer™ II Reagent could be thawed at room temperatures for several times without affecting RNA quantitative or quality.

New in this Edition:

1. This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	R0424-00	R0424-01	R0424-02
RNA safer™ II Reagent	5 mL	50 mL	250 mL
User Manual	✓	✓	✓

Storage and Stability

RNA safer™ II Reagent is stable for at least 24 months when stored at 15-25°C and yields reproducible results.

Before Beginning

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination.
- Use sterile, disposable plastic ware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase.
- Work quickly, but carefully.
- All centrifugation steps must be carried out at room temperature.
- Prepare all materials required before starting the procedure to minimize RNA degradation.
- Make sure that the samples remain submerged at all times during storage and transportation.

Handling Starting Material

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

Maximum Tissue Size

RNASafer™ II 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 contacts the samples. Therefore, samples size is critical for successful results. The ideal sample slices should be less than 0.4 cm thick.

RNASafer™ II Reagent Volume Estimation

In order to protect RNA, the surface of the tissue samples should be completely covered by RNASafer™ II Reagent. It is strongly recommended that the sample be put into at least 10 volumes of RNASafer™ II Reagent.

RNAsafer™ II Reagent Protocol

RNAsafer™ II Reagent Protocol - Total RNA Isolation from Tissue

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

1. Harvest tissue sample.
2. Cut the samples into smallest possible slices (< 0.4 cm).
3. Immediately immerse the sample into a container with 10 volumes of RNAsafer™ II Reagent.

Note: Make sure that the sample is fully covered by the reagent.

4. Take the sample out of RNAsafer™ II Reagent and cut proper amount. The remaining part could be put back to the storage. Remove RNAsafer™ II Reagent from sample using forceps and weigh (without affecting intactness of RNA in weighing time). Cut the sample into small pieces for easier lysis.
5. Estimate the amount of the tissue and select the appropriate E.Z.N.A.® Total RNA Kit. For Total RNA Kit I (R6834), use 15-20 mg tissue and add 500µL TRK Lysis Buffer. For E.Z.N.A.® Total RNA Kit II (Product R6934) or RNA-Solv® Reagent kit (Product R6830), use 15-20 mg stabilized tissue and 1 ml RNA Solv®. The volume of TRK Lysis Buffer could be adjusted according to the weight of tissue..
6. Following the user manual for the E.Z.N.A.® Total RNA system.

RNA Safer™ II Reagent Protocol

RNA Safer™ II Reagent Protocol - Total RNA Isolation from Cells

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

Materials and Equipment to be Supplied by User:

- Centrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes (Cat# SSI-1210-00)
- PBS

1. Centrifuge cells at 500 x g for 10 minutes.
2. Discard supernatant.
3. Wash cells with PBS.
4. Resuspend the cells with 10 volumes of RNA Safer™ II Reagent.
5. Centrifuge the cells at 5,000 x g for 5 min to remove the RNA Safer™ II. Alternatively, add one volume of cold PBS or sterile water to the cells and then centrifuge as above.
6. Select the appropriate E.Z.N.A.® Total RNA Kit. Use the Total RNA Kit I (R6834) for less than 1×10^7 cells, the Total RNA Kit II (Product R6934) or RNA-Solv® Reagent kit (Product R6830) for up to $5\text{-}10 \times 10^6$ eukaryotic cells or 1×10^8 bacterial cells, the Total RNA Midi Kit (R6664) for up to 1×10^8 eukaryotic cells or 1×10^{10} bacterial cells, or the Total RNA Maxi Kit (R6693) for up to 5×10^{10} cells.
7. Following the user manual for the E.Z.N.A.® Total RNA system.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Total RNA Kit I	R6834
Total RNA Kit II	R6934
RNA-Solv® Reagent Kit	R6830
Total RNA Midi Kit	R6664
Total RNA Maxi Kit	R6693

PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

