

E.Z.N.A.[®] RNA-Lock Reagent

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

February 2012

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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-Lock 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-Lock Reagent, tissue and cells are rapidly lysed and total cellular RNA is protected. Samples stored in RNA-Lock 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-Lock 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-Lock Reagent is suitable for small quantities of animal tissues (<150 mg), cultured cells, and white blood cells. The simplicity of the RNA-Lock Reagent method allows simultaneous processing of a large number of samples.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	R0424-00	R0424-01	R0424-02
RNA-Lock Reagent	5 mL	50 mL	250 mL
User Manual	✓	✓	✓

Storage and Stability

RNA-Lock Reagent is stable for at least 12 months when stored at 15-20°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 RNA-Lock Reagent, it is extremely important to treat the sample with RNA-Lock Reagent immediately after harvesting the material.

Maximum Tissue Size

RNA-Lock 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 0.25 cm thick.

RNA-Lock Reagent Volume Estimation

In order to protect RNA, the surface of the tissue samples should be completely covered by RNA-Lock Reagent. It is strongly recommended that the sample be put into at least 15 volumes of RNA-Lock Reagent.

RNA-Lock Reagent Protocol

RNA-Lock 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 (< 0.25 cm).
3. Immediately immerse the sample into a container with the appropriate volume of RNA-Lock Reagent.

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

4. Estimate the amount of the tissue and select the appropriate E.Z.N.A.® Total RNA Kit. Use the Total RNA Kit I (R6834) for less than 40 mg tissue, the Total RNA Midi Kit (R6664) for up to 200 mg tissue, or the Total RNA Maxi Kit (R6693) for 1 g tissue.
5. Following the user manual for the E.Z.N.A.® Total RNA system, homogenize the sample the maximum volume of TRK Lysis Buffer indicated in the protocol.
6. Clear the lysate by centrifugation as described in the protocol.
7. Add 1 volume 100% ethanol and transfer the sample to the column.
8. Continue to the next step in the user manual for the E.Z.N.A.® Total RNA system.

RNA-Lock Reagent Protocol

RNA-Lock 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 300 x g for 10 minutes.
2. Discard supernatant.
3. Wash cells with PBS.
4. Resuspend the cells with 50-100 μ L PBS.
5. Add 500 μ L to 1 mL RNA-Lock Reagent.
6. Transfer 150 μ L cells/RNA-Lock Reagent mixture to a RNase-free 1.5 mL microcentrifuge tube.
7. 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 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.
8. For the E.Z.N.A.[®] Total RNA Kit I, follow the protocol below. If using the E.Z.N.A.[®] Total RNA Midi Kit or Total RNA Maxi Kit, please follow the standard protocol in the user manual.

RNA-Lock Reagent Protocol

9. Add 200 μ L TRK Lysis Buffer. Mix thoroughly by pipetting up and down 3-5 times.

10. Add 350 μ L 70% ethanol. Mix thoroughly by pipetting up and down 3-5 times. Do not centrifuge.

11. Transfer the sample to the column.

12. Continue to the next step in 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 Midi Kit	R6664
Total RNA Maxi Kit	R6693
Nuclease-free 1.5 mL microcentrifuge tubes	SSI-1210-00

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

