# Total RNA Isolation from Cells and Tissues

## Contents

## Introduction

The EZgene™ Tissue RNA Kit plus combines the reversible binding properties of HiBind RNA technology with a specially designed buffer system which can effectively remove DNA before RNA isolation. Samples are first lysed and homogenized in a specially designed denaturing buffer, which immediately inhibits the activity of RNase. The lysate is then passed through a EZgene™ DNA Clearance column which traps the genomic DNA. The flow-through lysate is then applied to RNA column to bind RNA. After two wash steps, purified RNA is eluted with RNase-free water. The whole protocol can be completed in less than 30 minutes. RNA purified is ready for applications such as NGS, RT-PCR, qPCR, differential display, and microarrays.

## Storage and stability

All components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing.

## Kit contents

| **Catalog#** | **R6321-00** | **R6321-01** | **R6321-02** |
|---|---|---|---|
| **Preps** | 4 | 50 | 250 |
| Buffer HLY | 4 mL | 40 mL | 150 mL |
| Buffer HRB | 4 mL | 50 mL | 150 mL |
| RNA Wash Buffer | 2 mL | 12 mL | 50 mL |
| DEPC-Treated dd$H_2O$ | 1.0 mL | 10 mL | 30 mL |
| ezBind RNA Columns | 4 | 50 | 250 |
| DNA Clearance Column | 4 | 50 | 250 |
| Instruction Manual | 1 | 1 | 1 |

Caution: Buffer HLY and Buffer HRB contain chaotropic salts, wear gloves and protective eyewear when handling.

## Safety information

Buffer HLY contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

## Before starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

***Important***

- ☑ Calculate and aliquot amount of Buffer HLY to be used in a clean tube and add 10 µL β-mercaptoethanol (β-Me) or 40 µL DTT (1 M) to 1 mL Buffer HLY. Add 4 µL of L Solution per 1 mL of Buffer HLY/β-Me or Buffer HLY/DTT. Buffer HLY contains β-Me or DTT can be stored at room temperature up to 1 month.
- ☑ Crystals may form in Buffer HLY, dissolve the precipitates at 37 °C before use.
- ☑ Add 8 mL **(R6321-00)** or 48 mL (**R6321-01**) or 200 mL (**R6321-02**) 100% ethanol to RNA Wash Buffer before use. The final ethanol is 80% (v/v).

***Materials supplied by users***

- ☑ Tabletop microcentrifuge.
- ☑ Sterile RNase free 1.5 mL centrifuge tubes and tips.
- ☑ Vacuum manifold if use vacuum protocol.
- ☑ 14.3 M □-mercaptoethanol.

***Note: Perform all steps including centrifugation at room temperature. Carry out the procedure as quickly as possible to reduce the RNA degradation.***

## Disruption and homogenization of tissue samples

**It is critical to disrupt and homogenize the samples completely and properly for high quality RNA yield. The purpose for homogenization is to reduce the viscosity by shearing genomic DNA and other high molecular weight cell components to create a homogenous lysate. Incomplete homogenization may result in clogging the column and reducing the RNA yield.**

1. **Sample disruption by mortar and pestle**

- Excise tissues and freeze in liquid nitrogen immediate.
- Grind the sample with ceramic mortar and pestle to a fine powder under liquid nitrogen.
- Transfer the suspension into a tube pre-chilled in liquid nitrogen and allow the liquid nitrogen to evaporate while the samples remain frozen.
- Add Buffer HLY before the sample gets thawed.

2. **Homogenization using homogenization columns**

Up to 700 µL of samples can be loaded per column. Homogenization columns are supplied in the Plant RNA Kit and can be purchased separately for use with the tissue RNA kit.

3. **Rotor-Stator for sample disruption and homogenization**

Using a proper size probes and generator, the process simultaneously disrupts and homogenizes most of samples.

4. **Bead milling for sample disruption and homogenization**

Cells and tissues can be disrupted and homogenized by rapid agitation in the presence of **glass beads** in Buffer HLY. Use 4-8 mm glass beads for animal tissues, 0.5 mm for yeast cells and 0.1 mm for bacterial samples.

## Removal of genomic DNA using DNase digestion

For most of the downstream applications, DNase digestion is not necessary since the genomic DNA removal column and the RNA column remove nearly all genomic DNA. However, certain sensitive downstream applications may require further DNase I digest. Please reference Cat# E11-01 for DNase I digestion procedure.

## Stabilization of RNA in harvested animal tissues

The intact of RNA in harvested tissue will be protected with the addition of **RNASecure solution (Biomiga, catalog# R1011)**.

Cut the tissue into slices less than 0.5cm thick and immediately add at least 15 volumes of RNAsecure solution, for example, 150 µL RNAsecure solution per 10 mg tissue. Store at room temperature for up to 24 hours, at 4°C for up to a week, and -20°C or -80°C for long term.

## RNA quality

It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An $A_{260}/A_{280}$ ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

## Determine amounts of samples to be processed

| **Samples** | **Buffer HLY (500 µL)** | **Buffer HLY (700 µL)** |
|---|---|---|
| Cell numbers | = <5 x10^6 | >5x10^6-1x10^7 |
| Tissue mass | < 15 mg | >16-30 mg |

The yield depends on the tissue and cells to be processed. Please reference the table below to determine the amount of sample and expected yield.

| **Sample** | **10 mg/500 µL Buffer HLY** | **Total RNA Yield (µg)** |
|---|---|---|
| Liver | 10 mg | 50 (10 mg tissue) |
| Kidney | 10 mg | 20-30 (10 mg tissue) |
| Muscle* | 10 mg | 20 (10 mg tissue) |
| Spleen | 10 mg | 30-40 (10 mg tissue) |
| Heart* | 10 mg | 50 (10 mg tissue) |
| Lung | 10 mg | 10-20 (10 mg tissue) |
| Pancreas | 10 mg | 20 (10 mg tissue) |
| HeLa Cells | $1x10^6$ | 15 ($1x10^6$ cells) |
| 293HEK/ NIH/3T3 | $1x10^6$ | 10-12 ($1x10^6$ cells) |
| COS-7 | $1 x10^6$ | 30 ($1x10^6$ cells) |

# Protocol for extracting total RNA from cells

1. Cell preparations: (Scale up Buffer HLY if using more than $5x10^6$ of cells, do not use more than 1x10^7 cells as this exceeds the column capacity and cause genomic DNA contamination).
    - **Suspension cultured cells:** determine the cell numbers and collect cells by centrifuging at 300 x g for 1-3 min. Remove all supernatant completely by aspiration and proceed quickly to step 2. Work as quick as possible to reduce RNA degradation.
    - **Adherent cultured cells:** Determine cell numbers and aspirate the medium completely with a pasteur pipet. Go to step 2 immediately by adding Buffer HLY.

    **Note:** Supernatant must be removed completely. Residual supernatant may inhibit cell lysis and thus affect the RNA yield.

2. **Suspension cells:** Flicking the tube to loosen the cell pellet and add **500 µL Buffer HLY.**

    **Adherent cells:** Add **500 µL Buffer HLY** directly into the dish. Use pipet tip to mix and transfer the cell lysate to a 1.5 mL tube.
    **Note:** Determine the volume of Buffer HLY to be used and add 20 µL of □-mercaptoethanol (□-ME) per 1 mL Buffer HLY before use. Buffer HLY contains □-ME can be stored at room temperature for up to 1 month.

3. Homogenize the lysate by vortexing vigorously or repeated pipetting until the sample is uniformly homogenized.

4. Transfer the sample to a DNA clearance column and centrifuge at 13,000 rpm for 30s. Discard the DNA Clearance column and save the flow-through.

5. Add 0.5 **volume** of **100% ethanol** into the flow-through **(for example: 250 µL 100% ethanol** for **500 µL flow-through)**, mix thoroughly by pipetting up and down 10 times. Do not centrifuge.
6. Transfer the sample to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and re-use the collection tube.

7. Add **500 µL Buffer HRB** to the RNA column and centrifuge at 13,000 rpm for 30s. Discard the flow-through and put the column back to the collection tube.

8. Add **600 µL RNA Wash Buffer** *(Add ethanol before use)* to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through and put the column back to the collection tube.

9. Centrifuge the column at 13,000 rpm for 1 minute. It is critical to remove residual ethanol for optimized elution in next step.

10. Transfer the column to a RNase-free 1.5 mL tube and add **50-100 µL** RNA Elution buffer (**DEPC treated dd$H_2O$)** to the center of the column. Centrifuge at 13,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20 °C.

    Optional: Add the eluted RNA back to the column for another elution normally yields additional 20-30% of the RNA. The first elution normally yields 70-80% of the RNA.

## Protocol for animal tissue total RNA extraction

1. Quickly weight an appropriate mass tissue according to Table 1 (Page 6) and immediately transfer the tissue into a 1.5 ml tube containing **500 µL Buffer HLY** (add □-mercaptoethanol before use) and homogenize the tissue by a rotor starter or ultrasonic homogenizer on ice.

   **Note:** Determine the volume of Buffer HLY to be used and add 20 µL of □□mercaptoethanol (□□ME) per 1 mL Buffer HLY before use. Buffer HLY contains (□-ME) can be stored at room temperature for up to 1 month.

   **Note: Do not use over 30 mg of tissue per column as this causes incomplete tissue digestion and genomic DNA contamination.**

2. Centrifuge the lysate for 2 min at 13,000 rpm at room temperature and transfer the cleared lysate to a DNA Clearance column.

3. Centrifuge at 13,000 rpm for 30s. Discard the DNA Clearance column and save the flow-through.

4. Add 0.5 **volume** of **100% ethanol** into the flow-through **(for example: 250 µL 100% ethanol** for **500 µL flow-through)**, mix thoroughly by pipetting up and down 10 times. Do not centrifuge.

5. Transfer the solution to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and re-use the collection tube. Process the remaining sample.

6. Add **500 µL Buffer HRB** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through liquid and re-use the collection tube.

7. Add **600 µL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.
8. Centrifuge the column at 13,000 rpm 2 min. It is critical to remove residue ethanol for optimal elution in the following step.

9. Place the column to a RNase free 1.5 mL tube and add **50-100 µL** RNA Elution buffer (**DEPC treated dd$H_2O$)** to the column and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20$^{o}$C.

   Optional: Add the eluted RNA back to the column for another elution may yield additional 20-30% of the RNA. The first elution normally yields 70-80% of the RNA.

## Trouble shooting Guide

| Problems | Possible | Suggested Improvements |
|---|---|---|

| | reasons | |
|---|---|---|
| Low $A_{260}/A_{280}$ ratios | Protein contamination | Do Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected. |
| Low $A_{260}/A_{280}$ ratios | Guanidine Thiocyanate contamination | Add 2.5 volumes of ethanol and 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at –20°C. Centrifuge at 10,000 g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water. |
| Low Yield | RNA in sample degraded | Freeze samples immediately in liquid nitrogen and store at -80°C after collect it. |
| Low Yield | The binding capacity of the membrane in the spin column was exceeded | Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield. |
| Low Yield | Ethanol not added to buffer | Add ethanol to the RNA Wash Buffer and DNase Stop Solution before purification. |
| Genomic DNA contamination | Too much total RNA sample was used in RT-PCR. | Reduce total RNA amount used in RT-PCR to 50-100 ng. |
| Genomic DNA contamination | The sample may contain too much genomic DNA. | Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 30 mg or less per prep.<br><br>Reduce cell numbers to 1-2x$10^5$ or increase buffer volume and do multiple loadings to column. |

## Limited use and warranty

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

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