

EasyPrep™ Total RNA Extraction Miniprep Plus Manual

Catalog# R01-03, R01-04

For Purification of Total RNA From

- ♦ Cultured Cells
- ♦ Animal Tissues



For research use only. Not intended for diagnostic testing.

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Introduction

The EasyPrep™ Total RNA Extraction Miniprep Plus Kit provides an easy and fast method for isolating total RNA from tissues, cultured cells within 30 min. The genomic DNA in the cell lysate is removed by DNA columns. This kit purifies up to 100 µg of total RNA from eukaryotic cells or animal tissues. The purified RNA is ready for RT-PCR, Northern blotting, polyA⁺ RNA purification, nuclease protection, and *in vitro* translation.

Storage and Stability

The kit can be stored at room temperature. All kit components are guaranteed for 1 year from the date of purchasing.

Kit Contents

Catalog#	R01-03	R01-04
RNA Mini Columns	50	250
Buffer HLY	28 mL	135 mL
Buffer HRB	23 mL	135 mL
RNA Wash Buffer (concentrate)	12 mL	50 mL
DEPC-Treated H ₂ O	10 mL	30 mL
Genomic DNA Clearance columns	50	250
Collection Tubes	50	250

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

- ◆ Determine the volume of Buffer HLY to be used and add 20 µL of β-mercaptoethanol (β-ME) per 1 mL Buffer HLY before use. Buffer HLY contains β-ME can be stored at room temperature for up to 1 month.
- ◆ Crystals may form in Buffer HLY, dissolve the precipitates at 37°C before use.
- ◆ Add 48 mL (**R01-03**) or 200 mL (**R01-04**) 100% ethanol to RNA Wash Buffer before use. The final ethanol content is 80% (v/v).

Materials supplied by users

- ◆ Tabletop microcentrifuge and 1.5 mL sterile tubes.
- ◆ Vacuum manifold if use vacuum protocol.
- ◆ 100% ethanol.
- ◆ β-mercaptoethanol.

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 RNA Shredder columns

It is a fast and efficient way to homogenize the samples using Bioland's RNA shredder columns. Up to 700 µL of samples can be loaded per column, centrifuge at 11,000 rpm for 30s. Shredder columns 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.

Stabilization of RNA in Harvested Animal Tissues

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

1. 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.
2. 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 A260/A280 ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Determine Amounts of Samples to be Processed

The yield depends on the tissue and cells to be processed. Please reference Table 1 to determine the amount of sample.

Table 1. Typical yield of total RNA per column

Sample	10 mg/500 μ L Buffer HLY	Total RNA Yield (μ g)
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)
Brain**	10 mg	80 (10 mg tissue)
Lung	10 mg	10-20 (10 mg tissue)
Pancreas	10 mg	20 (10 mg tissue)
HeLa Cells	1×10^6	15 (1×10^6 cells)
293HEK	1×10^6	12 (1×10^6 cells)
COS-7	1×10^6	30 (1×10^6 cells)
NIH/3T3	1×10^6	10 (1×10^6 cells)

***Note:** It is normally difficult to isolate RNA from heart, muscle, and skin tissue using the regular RNA isolation procedure due to the rich contents of connective tissue, collagen, and contractile proteins. Optimization with the addition of proteinase K digestion that enables the removal of proteins described above is needed. We suggest you use **EasyPrep™ Tissue RNA Kit for heart, muscle, and skin tissue**.

****Note:** For isolating from lipid rich animal tissue, such as thymus and brain tissue, we recommend you use **EasyPrep™ Tissue RNA Kit for lipid-rich animal tissue**.

Extracting Total RNA From Cultured Cells

1. Cell preparations: (Do not use more than 5×10^6 of cells)

Suspension cultured cells: Collect cells by centrifuging at **300 x g** for **5 min**. Remove all supernatant completely by aspiration. Flicking the tube to loosen the cell pellet and add **500 μ L Buffer HLY** (add β -mercaptoethanol 20 μ L/ml before use).

Adherent cultured cells: Aspirate the medium completely with a Pasteur pipet. Add **500 μ L Buffer HLY** (add β -mercaptoethanol 20 μ L/ml before use) directly into the dish. Use pipet tip to mix and transfer the cell lysate to a 1.5 mL tube.

Note: Buffer HLY contains β -ME can be stored at room temperature for up to 1 month. Residual supernatant will inhibit cell lysis and affect the RNA yield.

- Homogenize the lysate by vortexing vigorously or repeated pipetting until the sample is uniformly homogenized.
- Centrifuge the solution at 11,000 rpm for 5 min at room temperature and transfer the clear lysate to a DNA Clearance column.
- Centrifuge at 11,000 rpm for 30 s. Discard the DNA Clearance column and save the flow-through.
- Add an equal volume of **100% ethanol** into the flow-through (for example: 500 μ L 100% ethanol for 500 μ L lysate); mix thoroughly by pipetting up and down 10 times. Do not centrifuge.
- Transfer the solution to a RNA column and centrifuge at 11,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.
- Add **400 μ L Buffer HRB** to the column and centrifuge at 11,000 rpm for 30s. Discard the collection tube with the flow-through.

8. Put the column back to a new collection tube. Add **500 µl RNA Wash Buffer** to the column and centrifuge at 11,000 rpm for 30 seconds. Discard the flow-through and put the column back to the collection tube. Repeat once.
9. Centrifuge the column with the lid open at 11,000 rpm for 1 min. It is critical to remove residue ethanol for optimal elution.
10. Transfer the column to an RNase-free 1.5 mL tube. Add **30-50 µL DEPC-treated ddH₂O** to the center of the column. Centrifuge at 11,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20 °C.

Extracting Total RNA From Animal Tissue

1. Quickly weigh an appropriate tissue mass according to Table 1 and transfer the tissue into a 1.5 ml tube. Add **500 µl Buffer HLY** (add b-mercaptoethanol 20 µl/ml before use) and homogenize the tissue by one of the methods on Page 4.
2. Centrifuge the solution at 11,000 rpm for 5 min at room temperature and transfer the clear lysate to a DNA Clearance column.
3. Centrifuge at 11,000 rpm for 30 s. Discard the DNA Clearance column and save the flow-through.
4. Add **an equal volume** of **100% ethanol** into the flow-through (for example: 500 µL 100% ethanol for 500 µL lysate); mix thoroughly by pipetting up and down 10 times. Do not centrifuge.
5. Transfer the solution to a RNA column and centrifuge at 11,000 rpm for 1 min. Discard the flow-through* and put the column back to the collection tube. *Keep the flow-through if protein is needed.
6. Add **400 µL Buffer HRB** to the column and centrifuge at 11,000 rpm for 30s. Discard the collection tube with the flow-through. Put the column back to a new collection tube. Keep the flow-through if protein is needed.
7. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 11,000 rpm for 30 s. Discard the flow-through. Repeat once. Ensure that ethanol has been added to RNA wash buffer before use.
8. Centrifuge the column with the lid open at 11,000 rpm for 1 min. It is critical to remove residue ethanol for optimal elution.
9. Transfer the column to an RNase-free 1.5 mL tube. Add **35-50 µl DEPC-treated ddH₂O** to the center of the column. Centrifuge at 11,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20 °C.

Troubleshooting

Problem	Possible Reasons	Suggested Improvement
Low A260/A280 ratios	Protein contamination	Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low A260/A280 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 11,000 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 -70°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. Reduce cell numbers to $1-2 \times 10^5$ or increase buffer volume and do multiple loadings to column.

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