EasyPrep™ Total RNA Extraction
Miniprep Plus Manual

Catalog#: R01-03, R01-04

For Purification of Total RNA From
- **Cultured Cells**
- **Animal Tissues**

Bioland

For research use only. Not intended for diagnostic testing.

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Contents

Introduction

The EasyPrep™ Tissue RNA Kit provides an easy and fast method for isolating total RNA from tissues or cultured cells within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment when it is necessary. 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

DNase I (optional) should be stored at -20^{o}C. All other components 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
DNA Mini Columns	50	250
Buffer HLY	28 mL	135 mL
Buffer HRB	23 mL	135 mL
5xRNA Wash Buffer	12 mL	54 mL
DEPC-Treated ddH2O	10 mL	30 mL
Collection Tubes	100	500

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 216 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 homogenization columns**
   It is a fast and efficient way to homogenize the samples using Bioland's RNA Shredders**.** 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

DNA digestion is necessary for downstream applications that are sensitive to very small amounts of DNA, for example, RT-PCR with low-abundance target. Generally, it is not required to do so since the EasyPrep™ RNA purification kit selectively isolates RNA and eliminates most of the DNA. If there is DNA contamination, either reduces the tissue amount or cells.

Stabilization of RNA in harvested animal tissues

The intact of RNA in harvested tissue will be protected with the addition of RNA Secure solution (*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 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)
Brain**	10 mg	80 (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	$1x10^6$	12 ($1x10^6$ cells)
COS-7	$1 x10^6$	30 ($1x10^6$ cells)
NIH/3T3	$1x10^6$	10 ($1x10^6$ 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.

****Note:** For isolating RNA from lipid rich animal tissue, such as thymus and brain tissue, the yield will be low.

Extracting total RNA from cultured cells

1. **Cell preparations: (Do not use more than 5x10^6 of cells)**

   Suspension cultured cells: **Collect cells by centrifuging at 300g for 5 min. Remove all supernatant completely by aspiration. Flicking the tube to loosen the cell pellet and add 500µl Buffer HLY** *(add b-mercaptoethanol 10 µl/ml before use).*

   Adherent cultured cells: **Aspirate the medium completely with a Pasteur pipet. Add 500µl Buffer HLY** *(add b-mercaptoethanol 10 µl/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.

2. **Homogenize the lysate by passing it through a 20-gauge needle fitted to an RNase and DNase free syringe for at least 5 times. If the solution is clear, go to step 4, otherwise go to step 3.**

3. **Centrifuge the solution at 13,000 rpm for 2 min and transfer the clear lysate to a clean 1.5 mL tube.**

4. **Transfer the clear cell lysate to a DNA column. Centrifuge at 13,000 rpm for 30s. Discard the DNA column and save the flow-through.**

5. **Add 0.7 Volume Acetone or Ethanol into the lysate and pipet 10 times to mix the solution. Vortex briefly if any precipitations.**

6. **Transfer the mixture to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.**

7. **Add 500 µl Buffer HRB to the column and centrifuge at 10,000 rpm for 30s. Discard the flow-through. Put the column back to the collection tube.**

8. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30s. Discard the collection tube with the flow-through. Put the column back to the collection tube.** *Ensure that ethanol is added to RNA Wash Buffer before use.*

9. **Add 450 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through. Put the column back to the collection tube. Centrifuge the column with the lid open at 13,000 rpm for 2 min.** *It is critical to remove residue ethanol for optimal elution.*

10. **Transfer the column to an RNase-free 1.5 mL tube. Add 50 µl DEPC-treated ddH_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.**

Extracting Total RNA from Animal Tissue

1. **Quickly weight an appropriate mass tissue according to Table 1 (Page 8) and transfer the tissue into a 1.5 ml tube containing 500 µl Buffer HLY (add β-mercaptoethanol before use) and homogenize the tissue by a rotor starter or ultrasonic homogenizer on ice.** *Determine the volume of Buffer HLY to be used and add 10 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. Use of too much mass of tissue per preparation will cause genomic DNA contamination.*
2. **Centrifuge the lysate for 5 min at 13,000 rpm at room temperature.**
3. **Transfer the clear cell lysate to a DNA column. Centrifuge at 13,000 rpm for 30. Discard the DNA column and save the flow-through.**
4. **Add 0.7 Volume Acetone or Ethanol into the lysate and pipet 10 times to mix the solution. Vortex briefly if any precipitations.**
5. **Transfer the mixture to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.**
6. **Add 500 µl Buffer HRB to the column and centrifuge at 10,000 rpm for 30s. Discard the flow-through. Put the column back to the collection tube.**
7. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30s. Discard the collection tube with the flow-through. Put the column back to the collection tube.** *Ensure that ethanol is added to RNA Wash Buffer before use.*
8. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through. Put the column back to the collection tube.**
9. **Centrifuge the column with the lid open at 13,000 rpm for 2 min.** *It is critical to remove residue ethanol for optimal elution.*
10. **Transfer the column to an RNase-free 1.5 mL tube. Add 50 µl DEPC-treated ddH_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.**

RNA cleaning Protocol

1. **Add 500 µl Buffer HLY** (add β-mercaptoethanol before use) **to the reaction (up to 100 µl).**
2. **Add 1/2 volume 100% ethanol into the mixture (for example: 250 µl 100% ethanol for 500 µl mixture) and pipet 5 times to mix the solution. Vortex briefly if any precipitations.**
3. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through. Repeat once.**
4. **Centrifuge the column with the lid open at 13,000 rpm for 1 min. Discard the flow-through.** *It is critical to remove residual ethanol for optimal elution.*
5. **Place the column to an RNase-free 1.5 ml tube. Add 50 µl DEPC-treated water to the column, incubate for 1 min, and centrifuge at 13,000 rpm for 2 min. Store the RNA solution at -20^{0}C.**

Troubleshooting

Problem	Possible reason	Suggested Improvement
Low A260/A280 ratios	Protein contamination	Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
	Guanidine 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 13,000 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.
	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.
	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 1-2x10^5 or increase buffer volume and do multiple loadings to column.

More EasyPrep™ Total RNA Purification Kits

Catalog #	Product Name	Preps
R01-01	Tissue RNA Miniprep kit	50
R01-02	Tissue RNA Miniprep kit	250
R9601-01	96-well Tissue RNA Miniprep kit	4x96
R9601-02	96-well Tissue RNA Miniprep kit	20x96
R02-01	Blood RNA Miniprep kit	50
R02-02	Blood RNA Miniprep kit	250
R9602-01	96-well Blood RNA Miniprep kit	4x96
R9602-02	96-well Blood RNA Miniprep kit	20x96
R03-01	Plant RNA Miniprep kit	50
R03-02	Plant RNA Miniprep kit	250
R9603-01	96-well Plant RNA Miniprep kit	4x96
R9603-02	96-well Plant RNA Miniprep kit	20x96
R1001-01	RNA Secure Solution	50 mL
R1001-02	RNA Secure Solution	100 mL

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland'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 Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland 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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