EasyPrep™ Blood Total RNA Extraction Miniprep Manual

Catalog#: R02-01, R02-02

Bioland

For research use only. Not intended for diagnostic testing.

(October 2013)

Contents

Introduction

The EasyPrep™ Tissue RNA Kit provides an easy and fast method for isolating total RNA from blood cells within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary. 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°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#	R02-01	R02-02
RNA Mini Columns	50	250
Buffer LY	28 mL	135 mL
Buffer RB	30 mL	135 mL
RNA Wash Buffer	12mL	2x54 mL
10x Red Blood Cell Lysis Solution	30 ml	135 ml
DEPC-Treated ddH2O	10 mL	30 mL
Collection Tubes	50	250
DNase I (Optional)	260 u (R01-05)	1300 u (R01-06)

Safety information

Buffer LY 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.

- Determine the volume of Buffer LY to be used and add 10 µL of β-mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains β -ME can be stored at 4°C.
- Crystals may form in Buffer LY, dissolve the precipitates at 37°C before use.
- Add 48 mL (**R02-01**) or 200 mL (**R02-02**) 100% ethanol to RNA Wash Buffer before use. The final ethanol content is 80% (v/v).
- Red blood lysis solution is supplied as 10x concentrate. Dilute with ddH_2O before use.

DNase I preparation

For each column, prepare the DNase I digestion reaction mixture as follows:

DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunits units/ µl)	1.5 µl
Total volume	75 µl

Materials supplied by users

- Tabletop microcentrifuge and 1.5 mL sterile tubes.
- Vacuum manifold if use vacuum protocol.
- 100% ethanol.
- β-mercaptoethanol.
- Optional: DNaseI, DNase buffer

Protocol For Total RNA Extraction From White Blood Cells (Leukocytes)

1. **Transfer 1-3 ml of whole blood (collected in heparinized or EDTA-treated tubes) into a 15 ml conical tube. Add 3 volumes of Red Blood Cell Lysis Solution and mix the solution by inversing the tube 5 times. Incubate on ice for 10 min** *Note: Dilute the 10x Red Blood Cell Lysis solution in ddH_2O before use.*
2. **Centrifuge the blood sample at 3,000 rpm (400 x g) for 5 min at 4°C. Remove the supernatant by carefully pipetting from the top of the sample. Do not disturb the precipitated blood cell pellet.**
3. **Transfer 500 µl Buffer LY to the leukocytes pellets and vortex the solution for 1 min.** *Ensure that β-mercaptoethanol has been added before use.*
4. **Centrifuge at 13,000 rpm for 5 min. Transfer the clear supernatant into a new RNase –free tube.**
5. **Add 0.5 volume 100% ethanol to the lysate (for example: 250 µl 100% ethanol for 500 µl lysate).**
6. **Transfer the solution into a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.**
7. **Add 500 µl Buffer RB to the column and centrifuge at 13,000 rpm for 30s. Discard the collection tube with the flow-through.**
8. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.** *Ensure that ethanol has been added to RNA Wash Buffer before use.*
9. **Optional: Add 75 µl DNase I (5U，RNase-free) solution onto the middle of the column and incubate at room temperature for 15 min. Add 200 µl Buffer RB to the column and incubate**

for 1-2 min, and centrifuge at 13,000 rpm for 30s. Discard the collection tube with the flow-through. Put the column back to a new collection tube.

10. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.**
11. **Centrifuge the column with the lid open at 13,000 rpm for 1 min.** It is critical to remove residue ethanol for optimal elution.
12. **Transfer the column to an RNase-free 1.5 mL tube. Add 35-50 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.**

Note: 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.

RNA cleaning Protocol

1. **Add 500 µl Buffer LY** (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. **Transfer the solution into the binding column and centrifuge at 10,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.**
4. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 10,000 rpm for 30 s. Discard the flow-through.**
5. ***Optional:*** **Add 75 µl DNase I (5U，RNase-free) solution onto the middle of the column and incubate at room temperature for 15 min. Add 400 µl Buffer RB onto the column and centrifuge at 10,000 rpm for 1 min. Discard the flow-through. Add 300 µl RNA Wash Buffer to the column and centrifuge at 10,000 rpm for 1 min. Discard the flow-through.**
6. **Add 500 µl RNA Wash Buffer to the column and centrifuge at 10,000 rpm for 30 s. Discard the flow-through.**
7. **Centrifuge the column with the lid open at 10,000 rpm for 1 min. Discard the flow-through.** It is critical to remove residual ethanol for optimal elution.
8. **Place the column to an RNase-free 1.5 ml tube. Add 35-50 µl DEPC-treated water to the column, incubate for 1 min, and centrifuge at 10,000 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 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 -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.

Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (877) 603-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

Visit our web at **www.bioland-sci.com** and learn more about Bioland products