Ver: 1903

DNA/RNA Multiprep Kit

（BW-DR3111）

Contents

Kit Contents

Catalog#	BW-DR3111-00	BW-DR3111-01	BW-DR3111-02
Preps	10	50	250
DNA Columns	10	50	250
2 mL Collection Tubes	10	50	250
RNA Columns	10	50	250
1.5 mL RNase-free Microfuge Tubes	10	50	250
Buffer HLY	12 mL	60 mL	260 mL
Buffer RB	12 mL	60 mL	260 mL
DNA Wash Buffer*	3 mL	12 mL	50 mL
RNA Wash Buffer*	3 mL	12 mL	50 mL
DNA Elution Buffer	1 mL	10 mL	25 mL
DEPC-treated ddH_2O	1 mL	10 mL	25 mL
User Manual	1	1	1

*Add 12 mL (BW-DR3111-00) or 48 mL (BW-DR3111-01) or 200 mL (BW-DR3111-02) 100% ethanol to RNA Wash Buffer and DNA Wash Buffer before use. The final ethanol is 80% (v/v).

Introduction

The EZgene™ DNA/RNA Multiprep Kit provides a simple and rapid method for simultaneously isolating genomic DNA and total RNA from a biological sample, which includes cultured cells, tissues, whole blood, plasma, serum, and body fluids. This co-purification system saves time on sample handling, avoids the sample variation and saves precious samples. It is essential when the amount of available samples is limited.

Storage and Stability

All components can be stored at room temperature (4-28℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

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

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/β-ME can be stored at room temperature (15-25℃) for up to 1 month.
- Crystals may form in Buffer HLY, dissolve the precipitates at 37℃ before use.
- Add 12 mL (BW-DR3111-00) or 48 mL (BW-DR3111-01) or 200 mL (BW-DR3111-02) 100% ethanol to RNA Wash Buffer and DNA Wash Buffer before use. The final ethanol is 80% (v/v).

Materials not Supplied

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

Note: Perform all steps including centrifugation at room temperature (15-25℃) . Carry out the procedure as quickly as possible to reduce the RNA degradation.

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 waste, wear gloves and protective eyewear when handling.

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-cooled 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.

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 $x10^6$	>5$x10^6$-1$x10^7$
Tissue mass	< 15 mg	>16-30 mg

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

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)

Protocol (for extracting DNA/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 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.
3. Homogenize the lysate by vortexing vigorously or repeat pipetting until the sample is uniformly homogenized.

RNA Isolation

4. Transfer the solution to a **DNA Column** and centrifuge at 12,000 rpm for 30 s. Save the **DNA Column** for genomic DNA purification.
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 solution to a **RNA Column** and centrifuge at 12,000 rpm for 1 min. Discard the flow through and put the column back to the collection tube.
7. Add **500 µL Buffer RB** to the **RNA Column** and centrifuge at 12,000 rpm for 30 s. Discard the flow-through liquid and reuse the collection tube.
8. Add **500 µL RNA Wash Buffer** to the **RNA Column** and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Put the **RNA Column** back to the collection tube and process the remaining sample till all solution has been passed through the **RNA Column**.

9. Centrifuge the **RNA Column** at maximum speed for 1 min. It is critical to remove residue ethanol for optimal elution in the following step.
10. Place the **RNA Column** to a **1.5 mL RNase-free Microfuge Tube** and add **50 µL DEPC-treated ddH_2O** to the column and centrifuge at 12,000 rpm for 30 s. The RNA is in the flow-through liquid. Store the RNA solution at -20℃.

Genomic DNA Isolation

11. Transfer the **DNA Column** from step **4** to a new **2 mL Collection Tube** and add **500 µL** of **Buffer RB**. Spin at 12,000 rpm for 1 min. Discard the flow though and put the column back to the collection tube.
12. Add **500 µL DNA Wash Buffer** and spin at 12,000 rpm for 1 min. Discard the flow through and put the column back to the collection tube.
13. Spin the **DNA Column** at maximum speed for 2 min. Transfer the **DNA Column** to a new 1.5 mL centrifuge tube, add **50 µL** of **DNA Elution Buffer**. Spin at 12,000 rpm for 1 min to elute the DNA.

Protocol (for extracting DNA/RNA from tissues)

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 β-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 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.

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

RNA Isolation

2. Centrifuge the lysate for 1 min at 12,000 rpm at room temperature and transfer the cleared lysate to a **DNA Column**. Centrifuge at 12,000 rpm for 1 min. Save the **DNA Column** for genomic DNA purification.
3. 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.
4. Transfer the solution to a **RNA Column** and centrifuge at 12,000 rpm for 1 min. Discard the flow through and put the column back to the collection tube.
5. Add **500 µL Buffer RB** to the **RNA Column** and centrifuge at 12,000 rpm for 30 s. Discard the flow-through liquid and re-use the collection tube.
6. Add **500 µL RNA Wash Buffer** to the **RNA Column** and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Put the column back to the collection tube and process the remaining sample till all solution has been passed through the **RNA Column**.
7. Centrifuge the **RNA Column** at maximum speed for 1 min. It is critical to remove residue ethanol for optimal elution in the following step.
8. Place the **RNA Column** to a **1.5 mL RNase-free Microfuge Tube** and add **50 µL DEPC-treated ddH_2O** to the column and centrifuge at 12,000 rpm for 30 s. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.

Genomic DNA Isolation

9. Transfer the **DNA Column** to a new **2 mL Collection Tube**, add **500 µL** of **Buffer RB** and spin at 12,000 rpm for 30 s. Discard the flow through and put the column back to the collection tube.
10. Add **500 µL** **DNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and put the column back to the collection tube.
11. Spin the column for 2 min at 12,000 rpm to dry the DNA membrane. Transfer the column to a 1.5 mL centrifuge tube and add **50 µL** of **DNA Elution Buffer**.
12. Spin at 12,000 rpm for 1 min to elute the DNA.

Trouble Shooting Guide

Problems	Possible reasons	Suggested improvements
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 0.1 M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at –20℃. Centrifuge at 10,000 × g for 15 min at 4℃. Resuspend the RNA pellet in DEPC-treated ddH_2O.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -80℃ 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 Buffer 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.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO 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.

For technical support or learn more product information, please contact us or visit our website.

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