USER GUIDE

HiYield™ Total RNA Extraction Kit

Ver. 2023 - 12

ARROWTEC

Precautions

I) Handling Requirements

Do not use a kit after its expiration date has passed.
Some reagents contain the hazardous compounds guanidine thiocyanate or guanidine hydrochloride . Do not let these reagents touch your skin, eyes, or mucous membranes.
If contact does occur, wash the affected area immediately with large amounts of water.
If you spill the reagents, dilute the spill with water before wiping it up.
Do not allow reagents containing guanidine thiocyanate to mix with sodium hypochlorite solution or strong acids. This mixture can produce a highly toxic gas.

II) Laboratory Procedures

Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Lysis Buffer or take appropriate measures according to local safety regulations. RBC Bioscience does not warrant that samples treated with Lysis Buffer are completely inactivated and non-infectious.
After sample processing is completed, remove and autoclave all disposable plastics, if you worked with potentially infectious sample material.
Do not eat, drink or smoke in the laboratory work area.
Do not pipette by mouth.
Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
Do not use sharp or pointed objects when working with the reagent cartridge, in order to prevent damage of the sealing foil and loss of reagent.
Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles.
Use the general precautions described in the literature.
Wash hands thoroughly after handling samples and test reagents.

III) Waste Handling

Discard unused reagents and waste in accordance with country, federal, state and local regulations

CONTENTS

Content and Storage

Total RNA Extraction Kit Mini

Blood/Bacteria/Cultured Cells

Cat.No. YRB50//YRB100

Store at room temperature 15 ℃~25 ℃

Kit Contents

Cat.No. YRB50

50 mini preps / kit

RBC Lysis Buffer.....120 ml
*RB Buffer**.....30 ml*
RT Buffer.....15 ml
R-W1 Buffer.....25 ml
R-Wash Buffer (Concentrated).....25 ml*
RNase-free Water.....10 ml
RB Column Set.....50 Sets
Filter Column Set.....50 Sets
(Comes with 2 ml Collection Tube)

Cat.No. YRB100

100 mini preps / kit

RBC Lysis Buffer.....120ml x 2
*RB Buffer**.....60 ml*
RT Buffer.....30 ml
R-W1 Buffer.....50 ml
R-Wash Buffer (Concentrated).....25 ml*
RNase-free Water.....10 ml
RB Column Set.....100 Sets
Filter Column Set.....100 Sets
(Comes with2 ml Collection Tube)

Sample (Protocols Included): *Whole Blood/Buffy Coat,* cultured animal cell, gram -ve/+ve bacteria.
Sample size: *minimun 300 µl of human whole blood,* 10^6 cultured mammalian cells, 10^8 bacterial cells.
For certain protocols items such as DNase I (Cat.RN050), Lysozyme may need to be purchased separately.

** Add 4 times volume (YRB50:100ml / YRB100:100 ml) of ethanol (96%~100%) to R-Wash Buffer before first use.*
*** ß-Mercaptoethanol (ß-ME) Must be added to RB Buffer before use. Add 10µl of ß-ME per 1 ml of RB Buffer. RB Buffer containing ß-ME can be stored at room temperature for up to 1 month.*
All components are RNase-free.

Content and Storage

Total RNA Extraction Kit Mini

Tissue/Cultured Cells

Cat.No. YRT50//YRT100

Kit Contents

Cat.No. YRT50

50 mini preps / kit

*RB Buffer**.....30 ml*
R-W1 Buffer.....25 ml
*R-Wash Buffer *.....25 ml*
RNase-free Water.....10 ml
Micropestles (RNase free): .....50 pcs
RB Column Set.....50 Sets
Filter Column Set.....50 Sets
(Comes with 2 ml Collection Tube)

Cat.No. YRT100

100 mini preps / kit

*RB Buffer**.....60 ml*
R-W1 Buffer.....50 ml
*R-Wash Buffer *.....25 ml*
RNase-free Water.....10 ml
Micropestles (RNase free): .....100 pcs
RB Column Set.....100 Sets
Filter Column Set.....100 Sets
(Comes with 2 ml Collection Tube)

Sample Sources (Protocols Included*): *Fresh/Frozen Animal Tissue,* cultured animal cell,
For certain protocols items such as DNase I (Cat.RN050), Lysozyme may need to be purchased separately.

** Add 4 times volume (YRT50:100 ml / YRT100:100ml) of ethanol to R-Wash Buffer before first use.*
*** ß-Mercaptoethanol (ß-ME) Must be added to RB Buffer before use. Add 10µl of ß-ME per 1 ml of RB Buffer. RB Buffer containing ß-ME can be stored at room temperature for up to 1 month.*
All components are RNase-free. .

Content and Storage

Total RNA Extraction Kit Mini

Plant

Cat.No. YRP50//YRP100

Kit Contents

Cat.No. YRP50

50 mini preps / kit

*RB Buffer**.....30 ml*
PRB Buffer.....30 ml
R-W1 Buffer.....25 ml
*R-Wash Buffer *.....25 ml*
RNase-free Water.....10 ml
RB Column Set.....50 Sets
Filter Column Set.....50 Sets
(Comes with 2 ml Collection Tube)

Cat.No. YRP100

100 mini preps / kit

*RB Buffer**.....60 ml*
PRB Buffer.....60 ml
R-W1 Buffer.....50 ml
*R-Wash Buffer *.....25 ml*
RNase-free Water.....10 ml
RB Column Set.....100 Sets
Filter Column Set.....100 Sets
(Comes with 2 ml Collection Tube)

Sample:*50 mg plant tissue*
Operation time:*< 60 min.*
Elution volume:*50 ml*
Yield:*5-30 µg*

**** Add 4 times volume (YRP50:100ml / YRP100:100ml) of ethanol (96%~100%) to R-Wash Buffer before first use.***

Additional Requirements: B-Mercaptoethanol (B-ME), DNase I (recommended).

***** ß-Mercaptoethanol (ß-ME) Must be added to RB Buffer before use. Add 10µl of ß-ME per 1 ml of RB Buffer. RB Buffer Containing ß-ME can be stored at room temperature for up to 1 month.***

Description

The Total RNA Extraction Kit is specially designed for purification of total RNA from bacterial, cultured cells and fresh
human whole blood. The method uses detergents and a chaotropic salt to lyse the cells and inactivate RNase.
Lysate is clarified with provided lysate filter columns. RNA in chaotropic salt solutions binds to the glass fiber matrix of the RB columns. Following
washing off of contaminants, the purified RNA is eluted by RNase-free water. ssRNA and dsRNA of > 200 bps to 1000's of bps in length are efficiently
purified. Purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

New and Improved

Total RNA Extraction Kits now including Lysate Filter columns for complete assurance of cell lysis.

Reference

Vogelstein, B ., and Gillespi e, D . (1979) Proc. Natl . Acad . Sci. USA 76, 615.

Note

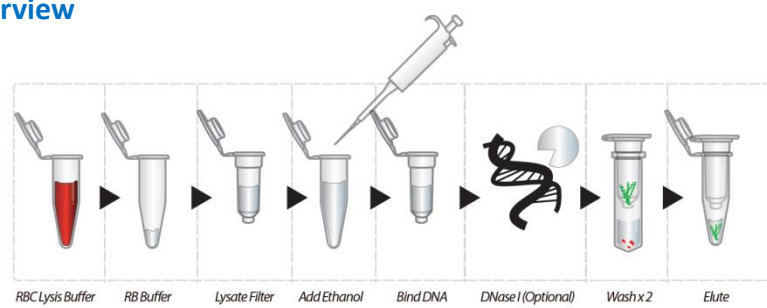
1. Total RNA Extraction Kit has been optimized for preferential RNA binding, however genomic DNA contamination is almost impossible to avoid during RNA extraction procedures. DNase I (RNase-Free) may be applied to the binding column according to the protocol instructions. It is necessary to use highly purified DNase. If RNase is present in trace amounts it will result in RNA degradation. It is recommended to apply DNase for sensitive downstream applications, however for many downstream applications it may not be necessary to apply as genomic DNA contamination may be neglibile or inconsequential to the application.

2. Wear a lab coat and disposable gloves to prevent RNase contamination.

3. Before use, add 10ml of ß-ME to 1mL of RB Buffer. RB Buffer Containing ß-ME can be stored at room temperature for up to 1 month.

Blood / Cultured Cells Protocol

Overview



Fresh Human Blood

RBC Lysis

1. Add 1 volume of human whole blood with 3 volumes of RBC lysis Buffer in an appropriately sized tube (not provided) and mix by inversion. Do not vortex. (For example, add 1.5 ml of RBC lysis Buffer to 500µl of whole blood. The minimum sample volume is 300µl.)
2. Incubate the tube for 10 minutes on ice and invert 2~3 times during incubation
3. Centrifuge for 3 minutes at 500 x g (2,500rpm) at 4˚C and completely discard the supernatant.
4. Add 500ul RBC lysis Buffer to the cell pellet. Resuspend cells by vortex briefly.
5. Centrifuge for 3 minutes at 500 x g (2,500rpm) at 4 ˚C and completely discard the supernatant.
6. Proceed to Lysis Step 1.

For Cultured Animal Cells

Cell Harvesting

If using adherent cells, trypsinize the cells before harvesting.

1. Transfer 10^6-10^7 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 1 min at 6,000 x g (8,000 rpm).
2. Proceed to Lysis Step 1.

Blood / Cultured Cells Protocol (continued)

Lysis

1. Add 400µl RB Buffer to the white pellet and mix by vortexing.
2. Incubate at room temperature for 5 min.
3. Place a Filter Column Set. Apply sample mixture to the column.
4. Centrifuge for 2 min at full speed (10,000 x g, 13,000 rpm).
5. Discard the Filter Column and transfer the clarified filtrate to a new microcentrifuge tube (not provided).

RNA Binding

6. Place a RB Column Set.
7. Add 400µl of 70% ethanol to the sample lysate and mix immediately by pipetting.
8. Apply the 500µl of the ethanol-added mixture to the RB Column.
9. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 2 minutes.
10. Discard the flow-through and apply the rest of the mixture to the same column.
11. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 2 minutes.
12. Discard the flow-through and place the RB Column back into the collection tube.

Optional:

If performing optional on-column DNase digestion (see “Recommended Step: DNA residue degradation” ,page 13) .

Proceed to Wash and RNA Elution Protocol (page.12) .

Bacterial Protocol

Additional Requirements (gram -ve): *RT Buffer*
(gram +ve): *Lysozyme Buffer (Not Provided)*

For Gram-negative Bacteria

1. Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (10,000 x g, 13,000 rpm) in a microcentrifuge and discard the supernatant. Vortex the cell pellet for 30 seconds.
3. Add 200µl of RT Buffer to the tube and vortex or pipette to resuspend the cell pellet.
4. Incubate at room temperature for 5 minutes.
5. Proceed to Cell Lysis Step1.

For Gram-positive Bacteria

Prepare Lysozyme Buffer: *(20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0), prepare the lysozyme buffer fresh immediately prior to use*.

1. Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided). Centrifuge the microcentrifuge tube for 1 min at full speed (10,000 x g, 13,000 rpm) and discard the supernatant.
2. Add 200µl of Lysozyme Buffer to the tube and vortex or pipette to resuspend the cell pellet.
3. Incubate at room temperature for 10 minutes. During incubation, invert tube every 2-3 minutes.
4. Proceed to Cell Lysis Step 1.

Cell Lysis

1. Add 400µl RB Buffer to the sample lysate and mix by vortexing.
2. Incubate at room temperature for 5 min.
3. Place a Filter Column Set. Apply sample mixture to the column
4. Centrifuge for 2 min at full speed (10,000 x g, 13,000 rpm).
5. Discard the Filter Column Set and transfer the clarifed filtrate to a new microcentrifuge tube (not provided).

RNA Binding

6. Place a RB Column Set.
7. Add 400µl of 70% ethanol to the sample lysate and mix immediately by pipetting.
8. Apply 500µl of ethanol-added mixture to the RB Column.
9. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 2 minutes.
10. Discard the flow-through and apply the rest of the mixture to the same column.
11. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 2 minutes.
12. Discard the collection tube containing the flow-through and place the RB Column back in Collection Tube.

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 13) .

Proceed to Wash and RNA Elution Protocol (page.12) .

Tissue Protocol

Addtional requirements:

- *20-G needle syringe*
- *70% Ethanol and 96% ~ 100% Ethanol.*
- *1.5 ml microcentrifuge tube (RNase-free).*
- *DNase I : Cat.No.:RN050*
- *ß - Mercaptoethanol (ß - ME): ß - ME must be added to RB Buffer before use. Add 10 µl of ß-ME per 1 ml of RB Buffer.*
- *RB Buffer is stable for 1 month at room temperature (15˚C ~ 25˚C) after addition of ß-ME.*

Cell Lysis

1. Cut off 10 mg of fresh or frozen animal tissue and transfer it into a RNase-free microcentrifuge tube (not provided).
2. Add 350 µl RB Buffer (ß-ME added) into the tube and use provided micropestle to sufficiently grind the tissue a few times.
3. Shear the tissue by passing lysate though a 20-G needle syringe 10 times.
4. Incubate at room temperature for 5 minutes. Place a Filter Column Set. Apply sample mixture to the column.
5. Centrifuge for 2 minutes at full speed (10,000 x g, 13,000 rpm) and transfer the clarified filtrate to a new microcentrifuge tube (not provided).
6. Add 350 µl of 70% ethanol to the sample lysate and mix immediately by pipetting.

RNA Binding

7. Place a RB Column Set.
8. Apply ethanol-added mixture from previous step to the RB Column.
9. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 2 minute.
10. Discard the flow-through and transfer the RB Column back in a 2 ml Collection tube.

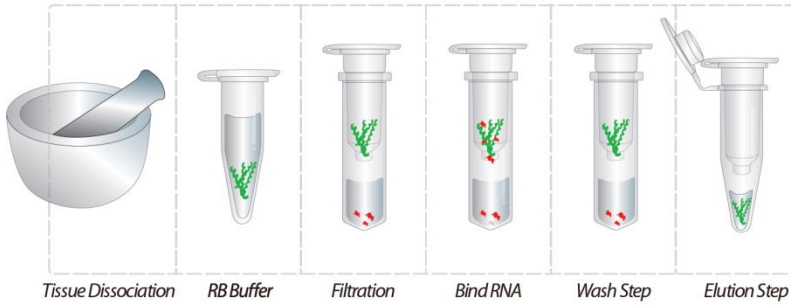
Optional:

If performing optional on-column DNase digestion (see “Recommended Step: DNA residue degradation” ,page 13) .

Proceed to Wash and RNA Elution Protocol (page.12) .

Plant Protocol (Duo 2 Buffer System)

Overview



Plant species are extremely diverse in their metabolic components. Large amounts of polysaccharides, polyphenolics , lipids and proteins may be distributed throughout the plant tissue. This special characteristic of plants means we offer two lysis buffers for optimum performance according to plant sample type.

RB: The standard protocol uses RB Buffer for lysis of plant samples. For most common plant species, this buffer system ensures purified RNA with high yields and little degradation.

PRB: An alternative buffer, PRB is also provided with the kit. The detergent system in this lysis buffer is suitable for plant samples containing large amounts of polysaccharides. In the majority of extractions both buffer systems should provide adequate results.

Tissue Dissociation

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder.
3. Transfer it into a microcentrifuge tube (not provided). Certain plant samples may not require liquid nitrogen treatment.

Lysis

4. Add 500 µl RB Buffer (or PRB) and 5 µl of ß-mercaptoethanol to ground sample and mix by vortexing.
5. Incubate at room temperature for 5 minutes.
6. Place a Filter Column Set. Apply sample mixture to the column.
7. Centrifuge for 2 minutes at full speed(10,000 x g, 13,000 rpm).
8. Discard the filter column and transfer the clarified filtrate to a new microcentrifuge tube (not provided).

Plant Protocol (Duo 2 Buffer System)(continued)

RNA Binding

9. Place a RB column Set.
10. Add a half of volume of 96 ~ 100 % ethanol to the sample lysate from Step 8 and mix immediately by vortexing. For example, add 250 µl of ethanol to 500 µl of filtrate.
11. Apply ethanol-added mixture from previous step to the RB column.
12. Centrifuge at full speed for 2 minutes.
13. Discard the flow-through and place the RB Column back in the 2ml Collection tube.

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 13) .

Proceed to Wash and RNA Elution Protocol (page.12) .

Wash and RNA Elution Protocol

Wash

1. Add 400 µl of R-W1 Buffer into the RB Column and centrifuge at full speed for 1 minute.
2. Discard the flow-through and place the RB Column back in the Collection Tube.
3. Add 600 µl of R-Wash Buffer (ethanol added) to the RB Column. Centrifuge at full speed for 1 minutes .
4. Discard the flow-through and place the RB Column back in the Collection Tube. Centrifuge at full speed for 3 minutes to dry the column matrix .

RNA Elution

5. Transfer dried RB Column to a clean microcentrifuge tube (RNase free, not provided).
6. Add 50µl of RNase free water in the Centre of the column matrix.
7. Allow to stand for 3 min until water is absorbed by the matrix.
8. Centrifuge at full speed for 1 min to elute purified RNA.

Recommended Step : DNA residue degradation (For Mini Column)

1. Add 200 µl Buffer R-W1 to the RB column. Close the lid gently, and centrifuge for 15s at full speed (10,000 x g, 13,000 rpm) to wash the spin column membrane. Discard the flow-through.
2. Follow either step 2a or 2b.

2a. Add 10 µl DNase I (Cat. No. RN050) stock solution (see RN050 manual) to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.
Buffer RDD is supplied with the RNase-Free DNase Set (Cat. No. RN050).
Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. **Do not vortex**.

2b. Add 20 Units DNase I stock solution (other brands) to 80 µl DNase reaction buffer.
(1M NaCl, 20mM Tris-HCl, 10mM $MnCl_2$, pH 7.0 at 25°C.)

3. Add the DNase I mixture (80 µl) directly to the RB column membrane, and incubate at 20–30°C for 15 min.
Note: Be sure to add the DNase I incubation mixture directly to the RB column membrane. DNase digestion will be incomplete if part of the mixture on the walls or the O-ring of the spin column.
4. Add 200 µl Buffer R-W1 to the RB column. Close the lid gently, and centrifuge for 15s at full speed (10,000 x g, 13,000 rpm). Discard the flow-through.

Continue with the Wash and RNA Elution Protocol, Wash step 3. (page 12)