Real Genomics

Store at room temperature 15°C~25°C

Total RNA Extraction kit Protocol Book

- **Blood/Bacteria/Cultured Cells** YRB50 // YRB100 // YRBM10 // YRM25
- **Tissue** YRT50 // YRT100
- **Plant** YRP50 // YRP100 // YRPM10 // YRPM25

Ver. 2013-01


RealGenomics

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

Total RNA Extraction Kit Mini

Blood/Bacteria/Cultured Cells

Cat.No. YRB50//YRB100

Store at room temperature 15°C~25°C

Kit Contents

Cat.No. YRB50

50 mini preps / kit

RBC Lysis Buffer	120ml
RB Buffer**	30ml
RT Buffer	15ml
R-W1 Buffer	25ml
R-Wash Buffer (Concentrated)*	25ml
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**	60ml
RT Buffer	30ml
R-W1 Buffer	50ml
R-Wash Buffer (Concentrated)*	25ml
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: minimun300µl of human whole blood, 10^6 cultured mammalian cells, 10^9 bacterial cells.
Yield: up to 30µg.
For complete genomic DNA removal , DNase I (RNase-free) must be added.

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*Add 4 times volume (YRB50:100/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 β-ME per 1 ml of RB Buffer. RB Buffer Contaning ß-ME can be stored at room temperature for up to 1 month.

All components are RNase-free.

Description

The Total RNA Extraction Kit (Blood/Bacterial/Cultured Cells) 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

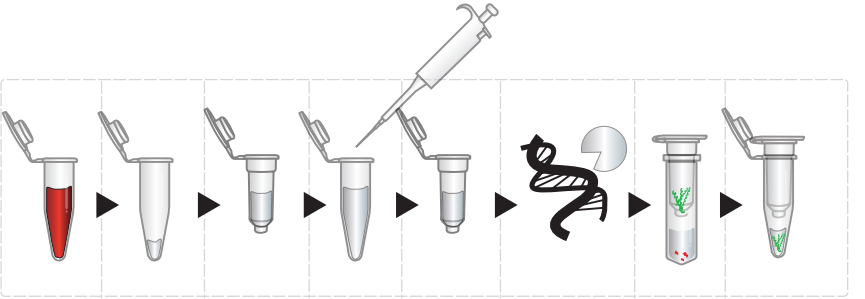
Total RNA Extraction Kits now including Lysate Filter columns for complete assurance of cell lysis.

Reference

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

Note

1. *Total RNA Extraction Kit has been optimised 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 columm according to the protocol instructions. It is neccessary 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 neccessary 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 Contaning ß-ME can be stored at room temperature for up to 1 month.*


RBC Lysis Buffer
RB Buffer
Lysate Filter
Add Ethanol
Bind DNA
DNase I (Optional)
Wash x 2
Elute

Blood / Cultured Cells Protocol

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℃ 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 ℃ 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. Discard the supernatant and resuspend the cells in 100µl PBS or RBC Lysis Buffer.
3. Proceed to Lysis Step 1.

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 41), follow procedure A.

Wash

13. Add 400µl of R-W1 Buffer into the RB Column. Centrifuge at full speed for 1 min.
14. Discard the flow-through and place the RB Column back in the Collection Tube.
15. Add 600µl of R-Wash Buffer (ethanol added) to the RB Column.
16. Centrifuge at full speed for 1 min.
17. Discard the flow-through and place the RB Column back in the Collection Tube.
18. Centrifuge at full speed for 3 minutes to dry the column matrix.

RNA Elution

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

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 41), follow procedure A.

Wash

13. Add 400µl of R-W1 Buffer into the Column. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 1 min.
14. Discard the flow-through and place the RB Column back in the Collection Tube.
15. Add 600µl of R-Wash Buffer (ethanol added) to the RB Column. Centrifuge at full speed for 1 min.
16. Discard the flow-through and place the RB Column back in the Collection Tube.
17. Centrifuge at full speed for 3 min to dry the column matrix.

RNA Elution

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

Quality Control

The quality of Total RNA Mini Kit (Blood/Cultured Cells) is tested on a lot-to-lot basis. The Kits are tested by isolation of total RNA from 300µl of fresh human whole blood. More than 1µg of total RNA was quantified with a spectrophotometer and checked by formaldehyde agarose gel analysis.

Total RNA Extraction Kit Maxi

Blood/Cultured Cells/Tissue/Bacteria

Cat.No. YRBM10//YRBM25

Store at room temperature 15°C~25°C

Kit Contents

Cat.No. YRBM10

10 Maxi preps / kit

RBC Lysis Buffer	200ml x2
RB Buffer**	60ml
RT Buffer	30ml
R-W1 Buffer	50ml
R-Wash Buffer *	25ml
RNase-free Buffer	10ml
RB Maxi Column Set	10 Sets
Maxi Filter Column Set	10 Sets

(Comes with 50ml Centrifuge Tube)

Cat.No. YRBM25

25 maxi preps / kit

RBC Lysis Buffer	500ml x2
RB Buffer**	150ml
RT Buffer	60ml
R-W1 Buffer	130ml
R-Wash Buffer *	40ml
RNase-free Buffer	30ml
RB Maxi Column Set	25 Sets
Maxi Filter Column Set	25 Sets

(Comes with 50ml Centrifuge Tube)

Sample (Protocols Included): Smaple100~200 mg animal tissue, 5 ml blood sample
Yield: up to 500 µg.
Operationtime: < 60 min
Elution volume: 500 µl
For complete genomic DNA removal DNase I (RNase-free) must be added.

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* Add 4 times volume 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 Contaning ß-ME can be stored at room temperature for up to 1 month.

All components are RNase-free.

Description

Total RNA Extraction Maxi Kit provides a fast and simple method to isolate total RNA from blood, bacteria, cultured tissue and cells. in the process, sample is ground in liquid nitrogen and filtrated by filter column to remove cell debris. In the presence of binding buffer with chaotropic salt, the total RNA in the lysate binds to glass fiber matrix in the RB Maxi Column. The optional DNase treatments can remove DNA residues and the contaminants are washed with an ethanol contained Wash Buffer. Finally, the purified total RNA is eluted by RNase-Free Water. The protocol does not require phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 min. The purified total RNA is ready for RT, RT-PCR, Real-time PCR, and Northern blotting.

Quality Control

The quality of Total RNA Maxi Kit is tested on a lot-to-lot basis.

Caution

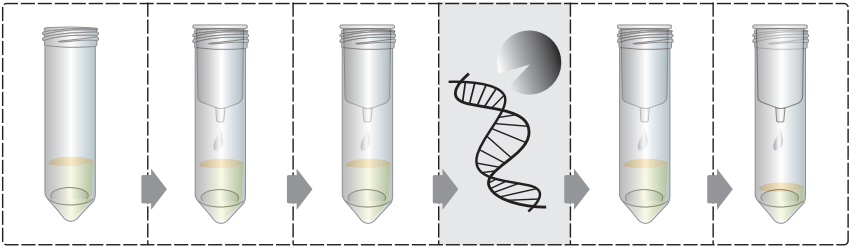
The component contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles. Always try to operate in an RNase free environment.

Notes on DNase I treatment and co-purification

When extracting RNA, it is normal to expect co-extraction of some genomic DNA as they are chemically very similar. It can be observed positioned above the RNA on an agarose gel. This is acceptable according to certain applications. If pure RNA is required, it will be neccessary to perform DNase I (RNase-free) treatment. Prepare the DNase I in reaction buffer according to manufacturer's instructions.

Apply to column according to instructions in protocol below. Highly purified RNase-free DNase I is neccessary to prevent RNA degradation. If experiencing larger amounts of genomic DNA contamination or extremely pure RNA is required, add more DNase or increase the incubation time of this step. However this may reduce overall RNA yield.

Blood Protocol (Use fresh humen blood)



Lysis

1. Collect fresh human blood in anticoagulant-treated collection tubes.
2. Add 15ml RBC Lysis Buffer to a sterile 50ml centrifuge tube (not provided).
3. Add 5 ml human whole blood and mix by inversion. Do not vortex.
4. Incubate the tube for 10min at room temperature.
5. Centrifuge for 5 min at 500 × *g* .
6. Discard the supernatant.
7. Add 5 ml RB Buffer and 50 µl of β-Mercaptorthanol (not provided) to sample and mix by votexing.
8. Place a Filter Maxi Column Set. Apply sample mixture to the column.
9. Centrifuge for 5 minutes at full speed.
10. Discard the Filter Maxi Column and transfer the clarified filtrate to a new 15ml Centrifuge Tube (not provided).

RNA Binding

11. Place a RB Maxi Column Set.
12. Add half sample volume of 96 ~ 100% ethanol to the sample lysate from Step10 and mix immediately by vortexing. For example, add 2.5 ml of ethanol to 5 ml of filtrate.
13. Apply ethanol-added mixture from previous step to the RB Maxi Column .
14. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 42), follow procedure B.

Wash

15. Add 4 ml of R-W1 Buffer into the column.
16. Centrifuge at full speed for 3 minutes.
17. Discard the flow-through and place the RB Maxi Column back in the 50ml Centrifuge Tube.
18. Add 6 ml of R-Wash Buffer (ethanol added) into the column.
19. Centrifuge at full speed for 3 minutes.
20. Discard the flow-through and place the RB Maxi Column back in the 50ml Centriguge Tube.
21. Centrifuge at full speed for 10 minutes to dry the column matrix.

RNA Elution

22. Place dried RB Maxi Column in a clean 50 ml centrifuge tube (RNase-free, not provided).
23. Apply 500µl of RNase-free water into the center of the column matrix.
24. Stand for 5 minutes until water is absorbed by the matrix.
25. Centrifuge at full speed for 5 minute to elute purified RNA.

Cultured Cells Protocol (Suspension cultured animal cells)

Trypsinize Cells

Adherent cultured cells: If using adherent cultured cells, trypsinize cells before lysis or lyse cells directly in culture dish.

a. *Remove medium, and wash cells with PBS.*
b. *Aspirate PBS and add 0.10-0.25% trypsin in PBS to trypsinize the cells.*
c. *After cells detach, add medium and transfer cells to 15ml centrifuge tube (not provided).*
d. *Pellet cells as Suspension cultured animal cells.*

Lysis /Cell Harvesting

1. *Transfer 10^7-10^8 of cells to a 15 ml centrifuge tube (not provided) and havest the cells with centrifugation for 5 minutes at 300 x g.*
2. *Remove the supernatant .*
3. *Add 5 ml RB Buffer and 50 µl of β-mercaptoethanol (not provided) to ground sample and mix by vortexing.*
4. *Place a Filter Maxi Column Set. Apply sample mixture to the column.*
5. *Centrifuge for 5 minutes at full speed.*
6. *Discard the Filter Maxi Column and transfer the clarified filtrate to a new 15ml centrifuge tube (not provided).*
7. *Processd to RNA Binding Step 16.*

To Lyse Cells in Culture Dish/ Flask.

8. Remove culture medium.
9. Add 5 ml RB Buffer and 50 µl of β-mercaptoethanol(not provided) to Culture dish or flask.

Important : Fully cover the cells with RB Buffer in dish/flask by shaking.

10. Collect cell lysate with a rubber policeman.
11. Place a Filter Maxi Columnin a 50ml Centrifuge Tube.
12. Apply sample lysate to the column.
13. Centrifuge for 5 minutes at full speed.
14. Discard the Filter Maxi Column and transfer the clarified filtrate to a new 15ml Centrifuge Tube (not provided)

RNA Binding

15. Place a RB Maxi Coumn Set.
16. Add a half of volume of 96 ~100% ethanol to the sample lysate from Step 6 or 15 and mix immediately by votexing. For Example, add 2.5ml of ethanol to 5 ml of filtrate.
17. Apply ethanol-added mixture from previous step to the RB Maxi Column.
18. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 42), follow procedure B.

Wash

19. Add 4 ml of R-W1 Buffer into the column.
20. Centrifuge at full speed for 3 minutes.
21. Discard the flow-through and place the RB Maxi Column back in the 50ml Centrifuge Tube.
22. Add 6 ml of R-Wash Buffer (ethanol added) into the column.
23. Centrifuge at full speed for 3 minutes.
24. Discard the flow-through and place the RB Maxi Column back in the 50ml Centriguge Tube.
25. Centrifuge at full speed for 10 minutes to dry the column matrix.

RNA Elution

26. Place dried RB Maxi column in a clean 50 ml centrifuge tube (RNase-free, not provided).
27. Apply 500µl of RNase-free water into the center of the column matrix.
28. Allow to Stand for 5 minutes until water is absorbed by the matrix.
29. Centrifuge at full speed for 5 minute to elute purified RNA.

Bacterial Protocol

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

For Gram-negative Bacteria

1. Transfer bacterial culture (10^{9}~10^{10}) to a 15 ml centrifuge tube (not provided).
2. Centrifuge for 5 min at full speed and discard the supernatant.
3. Add 2ml of RT Buffer to the tube and vortex or pipette to resuspend the cell pellet.
4. Incubate at room temperature for 5 minutes.

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}~10^{10}) to a 15ml centrifuge tube (not provided).
2. Centrifuge for 5 min at full speed and discard the supernatant.
3. Add 2ml of Lysozyme Buffer to the tube and vortex or pipette to resuspend the cell pellet.
4. Incubate at room temperature for 10 minutes During incubation, invert the tube every 2-3 min.

Lysis

5. Add 5 ml RB Buffer and 50 µl of β-me (not provided) to ground sample and mix by vortexing.
6. Incubate at room temperature for 5 min.
7. Place a Filter Maxi Column Set. Apply sample mixture to the column
8. Centrifuge for 5 min at full speed .
9. Discard the Filter Column Set and transfer the clarifed filtrate to a new 15ml centrifuge tube (not provided).

RNA Binding

10. Place a RB Maxi Column Set.
11. Add a half of volume of 96 ~100% ethanol to the sample lysate from Lysis Step and mix immediately by vortexing. For example, add 2.5 ml of 96 ~ 100% ethanol to 5 ml of filtrate
12. Apply ethanol-added mixture from previous step to the RB Maxi Column.
13. Centrifuge at full speed for 5 minutes and discard the flow-through

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 42), follow procedure B.

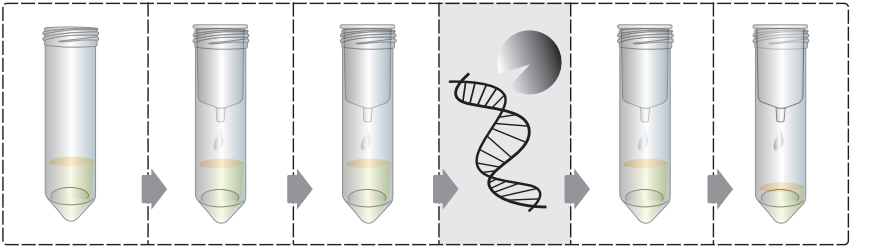
Wash

14. Add 4 ml of R-W1 Buffer into the column.
15. Centrifuge at full speed for 3 minutes.
16. Discard the flow-through and place the RB Maxi Column back in the Centrifuge Tube.
17. Add 6 ml of R-Wash Buffer (ethanol added) to the RB Maxi Columm. Centrifuge at full speed for 3 min.
18. Discard the flow-through and place the RB Maxi Column back in the Collection Tube.
19. Centrifuge at full speed for 10 minutes to dry the columm matrix.

RNA Elution

20. Transfer dried RB Maxi Column to a clean microcentrifuge tube (RNase-free, not provided).
21. Apply 500µl of RNase-free water in the center of the column matrix.
22. Allow to Stand for 5 minutes until water is absorbed by the matrix.
23. Centrifuge at full speed for 5 minutes to elute purified RNA.

Tissue Protocol



Lysis

1. Cut off 100 mg (up to 200mg) of fresh or frozen animal tissue.
2. Grind the sample under liguid nitrogen to a fine power.
3. Add 5 ml RB Buffer and 50 µl of β-Mercaptorthanol (not provided) to sample and mix by votexing.
4. Transfer it into a 15 ml centrifuge tube (not provided).
5. Incubate at room temperature for 5 minutes.
6. Place a Maxi Filter Column in a 50ml Centrifuge Tube. Apply sample mixture to the column.
7. Centrifuge for 5 minutes at full speed
8. Discard the Maxi Filter Column and transfer the clarified filtrate to a new 15ml Centrifuge Tube (not provided).

RNA Binding

9. Place a RB Maxi Column in a 50ml Centrifuge Tube.
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 2.5 ml of ethanol to 5 ml of filtrate.
11. Apply ethanol-added mixture from previous step to the RB-Maxi Column.
12. Centrifuge at full speed for 5 minutes and discard the flow-through.

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 42), follow procedure B.

Wash

13. Add 4 ml of R-W1 Buffer into the column.
14. Centrifuge at full speed for 3 minutes.
15. Discard the flow-through and place the RB Maxi Column back in the 50 ml Centrifuge Tube.
16. Add 6 ml of R-Wash Buffer (ethanol added) to the RB Maxi Columm. Centrifuge at full speed for 3 min.
17. Discard the flow-through and place the RB Maxi Column back in the 50 ml Centrifuge Tube.
18. Centrifuge at full speed for 10 minutes to dry the columm matrix.

RNA Elution

19. Transfer dried RB Maxi column to a clean microcentrifuge tube (RNase-free, not provided).
20. Add 500µl of RNase-free water in the center of the column matrix.
21. Allow to Stand for 5 minutes until water is absorbed by the matrix.
22. Centrifuge at full speed for 5 minutes to elute purified RNA.

Total RNA Extraction Kit Mini

Tissue

Cat.No. YRT50//YRT100

Kit Contents

Cat.No. YRT50

50 mini preps / kit

RB Buffer**	30ml
R-W1 Buffer	25ml
R-Wash Buffer*	25ml
RNase-free Water	10 ml
Micropestles (RNase free):	50pcs
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**	60ml
R-W1 Buffer	50ml
R-Wash Buffer*	25ml
RNase-free Water	10 ml
Micropestles (RNase free):	100pcs
RB Column Set	100 Sets
Filter Column Set	100 Sets

(Comes with 2 ml Collection Tube)

Sample Sources (Protocols Included*): Fresh/Frozen Animal Tissue.

* For certain protocols items such as DNase I, Lysozyme may need to be purchased separately

* Add 4 times volume (YRT50:100/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 β-ME per 1 ml of RB Buffer. RB Buffer Contaning ß-ME can be stored at room temperature for up to 1 month.

All components are RNase-free.

Description

The Total RNA Extraction Kit Mini (Tissue) is specially designed for purification of total RNA from a variety of animal tissues or cells. The provided micropestle efficiently homogenizes tissue samples in a microcentrifuge tube. The method uses detergents and a chaotropic salt to lyse cell and inactivate RNase. Lysate is clarified with provided lysate filter columns. RNA in the chaotropic salt solution is bound to the glass fiber matrix of the RB column. Following washing off of the contaminants the purified RNA is eluted by RNase-free water. Purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

Quality Control

The quality of Total RNA Extraction Kit Mini (Tissue) is tested on a lot-to-lot basis.

Notes

Total RNA Extraction Kit has been optimised 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 neccessary 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 neccessary to apply as genomic DNA contamination may be neglibile or inconsequential to the application.

Reference

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

Note

* *Use sterile, RNase-free pipet tips and microcentriuge tube. * Wear a lab coat and disposable gloves to prevent RNase contamination.*

Tissue Protocol

Addtional requirements:

- 20-G needle syringe
- 70% Ethanol and 96% ~ 100% Ethanol.
- 1.5 ml microcentrifuge tube (RNase-free).
- DNase I : RBC 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 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 41), follow procedure A.

Wash

11. Apply 400 µl of R-W1 Buffer into the column.

12. Centrifuge at full speed for 1 minute.

13. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.

14. Add 600 µl of R-Wash Buffer (96 -100% ethanol added) into the column.

15. Centrifuge at full speed for 1 minute.

16. Discard the flow-though and place the RB Column back in the 2 ml Collection Tube.

17. Centrifuge at full speed (10,000 x g, 13,000 rpm) for 3 minutes to dry the column matrix.

RNA Elution

18. Place dried RB Column in a clean microcentrifuge tube (RNase-free, not provided).

19. Add 50 µl of RNase-free water into the center of the column matrix.

20. Stand for 3 minutes until RNase-free water is absorbed by the matrix.

21. Centrifuge at full speed for 1 minute to elute purifed RNA.

Total RNA Extraction Kit Mini

Plant

Cat.No. YRP50//YRP100

Kit Contents

Cat.No. YRP50

50 mini preps / kit

RB Buffer**	30ml
PRB Buffer	30ml
R-W1 Buffer	25ml
R-Wash Buffer *	25ml
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**	60ml
PRB Buffer	60ml
R-W1 Buffer	50ml
R-Wash Buffer *	25ml
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
Operationtime: < 60 min.
Elution volume: 50 ml
Yield: 5-30 µg

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* Add 4 times volume (YRP50:100/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 β-ME per 1 ml of RB Buffer. RB Buffer Contaning ß-ME can be stored at room temperature for up to 1 month.

Description

The Plant Total RNA Mini Kit provides a fast and simple method to isolate total RNA from plant tissue and cells. In this process the sample is first ground in liquid nitrogen and filtrated by filter column to remove cell debris. In the presence of binding buffer and a chaotropic salt the total RNA in the lysate binds to the glass fiber matrix in the spin column. The optional DNase treatments can remove DNA residues and contaminants are washed out with an ethanol based wash buffer. Finally, the purified total RNA is eluted by RNase - free water. The protocol does not require phenol extraction and Ethanol precipitation. The entire procedure can be completed in 60 minutes. The purified total RNA is ready for RT, RT-PCR, real-time PCR, Northern blotting.

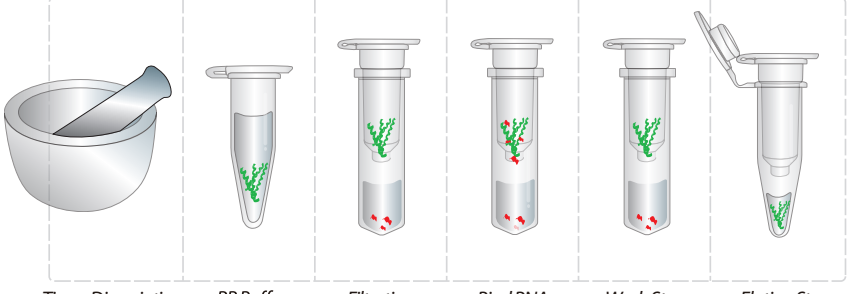
Quality Control

The quality of Plant Total RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 25 mg of young leaves. Purified RNA is quantified and checked by agarose gel electrophoresis.

Caution

The components contain an irritant agent. During use, always wear a lab coat disposable gloves, and protective goggles.

Plant Protocol (Duo 2 Buffer System)



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 sanples 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).*

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 41), follow procedure A.

Wash

14. Add 400 µl of R-W1 Buffer into the RB Column and centrifuge at full speed for 1 minute.
15. Discard the flow-through and place the RB Column back in the Collection Tube.
16. Add 600 µl of R-Wash Buffer (ethanol added) to the RB Column. Centrifuge at full speed for 1 minutes .
17. 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

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

Total RNA Extraction Kit Maxi
Plant

Cat.No. YRPM10//YRPM25

Kit Contents

Cat.No. YRPM10
10 maxi preps / kit

RB Buffer	60ml
PRB Buffer	60ml
R-W1 Buffer	50ml
R-Wash Buffer *	25ml
RNase-free Water	10 ml
RB Maxi Column Set	10 Sets
Filter Maxi Column Set	10 Sets

(Comes with 50ml Centrifuge Tube)

Cat.No. YRPM25
25 maxi preps / kit

RB Buffer	150ml
PRB Buffer	150ml
R-W1 Buffer	130ml
R-Wash Buffer *	40ml
RNase-free Water	30 ml
RB Maxi Column Set	25 Sets
Filter Maxi Column Set	25 Sets

(Comes with 50ml Centrifuge Tube)

Sample: 500 mg
Yield: 50-300µg
Time: <60 Mins
Elution Volume: 500µl

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* Add 4 times volume (YRPM10:100/YRPM25:160ml) ethanol (96%~100%) to R-Wash Buffer before first use.
Additional Requirements: ß-Mercaptoethanol (ß-ME), 15ml/50ml centrifuge tube, DNase I (recommended)

Description

The Total RNA Extraction Maxi Kit (Plant) provides a fast and simple method to isolate total RNA from plant tissue and cells. In this process the sample is first ground in liqid nitrogen and filtrated by filter column to remove cell debris. In the presence of binding buffer and a chaotropic salt the total RNA in the lysate binds to the glass fiber matrix in the spin column. The optional DNase treatments can remove DNA residues and contaminations are washed out with an ethanol based wash buffer. Finally, the purified total RNA is eluted by RNase-free water.

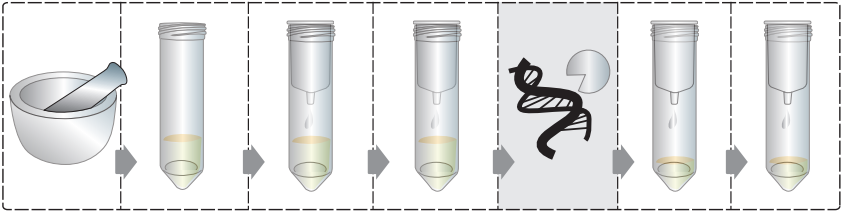
The protocol doesn't require phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes. The purified total RNA is ready for RT, RT-PCR, Real-Time PCR and Northern blotting. Plant species are extremely diverse in their metabolic components. Large amounts of polysaccharides, carbohydrates, lipids, poly-phenols and proteins may be distributed throughout the plant tissue. Duo to this special characteristic of plants, we offer two lysis buffers for optimum performance depends on different plant sample types.

RB:

The standard protocol uses RB Buffer for plant sample lysis. 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 present in this buffer is more effective in dispersing plant samples with large amounts of polysaccharide. In the majority of extractions, both buffer systems should provide adequate results. Researchers may try one buffer system first or both in parallel.


Tissue Dissociation
Lysis Buffer
Bind RNA
Wash
DNase I (Optional)
Wash x 2
Elute

Plant Maxi Protocol

Tissue Dissociation

1. Cut off 500 mg (up to 1g) of fresh or frozen plant tissue.
2. Grind the sample with mortar and pestle under liquid nitrogen to a fine powder.
3. Transfer it into a 15ml centrifuge tube (not provided). For some plant samples, liquid nitrogen may be unnecessary for homogenization.

Lysis

4. Add 5 ml RB Buffer (or PRB) and 50 µl β-mercaptoethanol to ground sample and mix by votexing.
5. Incubate at room temperature for 5 minutes.
6. Place a Filter Maxi Column Set. Apply sample mixture to the column.
7. Centrifuge for 5 minutes at full speed.
8. Discard the Filter Maxi Column and transfer the clarified filtrate to a new 15 ml centrifuge tube (not provided)

RNA Binding

9. Place a RB Maxi Column Set.
10. Add a half volume of 96 ~ 100% ethanol to the sample lysate and mix immediately by vortexing.
11. Apply ethanol-added mixture from previous step to the RB-Maxi Column. Centrifuge at full speed for 5 minutes and discard the flow-through..

Optional:

If performing optional on-column DNase digestion (see "Recommended Step: DNA residue degradation", page 42), follow procedure B.

Wash

12. Add 4 ml of R-W1 Buffer into the RB Maxi Column.
13. Centrifuge at full speed for 3 minutes.
14. Discard the flow-through and place the RB Maxi Column back in the Centrifuge Tube.
15. Add 6 ml of R-Wash Buffer (ethanol added) into the RB Maxi Column.
16. Centrifuge at full speed for 3 minutes.
17. Discard the flow-through and place the RB Maxi Column back in the Centrifuge Tube
18. Centrifuge at full speed for 10 minutes to dry out the column matrix.

RNA Elution

19. Place dried RB Maxi Column into a clean 50 ml Centrifuge Tube (RNase-free, not provided)
20. Add 500 µl of RNase-free water into the center of the column matrix.
21. Stand for 5 minutes until the water is absorbed by the matrix.
22. Centrifuge at full speed for 5 minutes to elute purified DNA.

Recommended Step : DNA residue degradation

Procedure A (For Mini Column)

1. Add 200 µl Buffer R-W1 to the RB column. Close the lid gently, and centrifuge for 15 s 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 (RBC 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 (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.

(1MNacl, 20mM Tris-HCl, 10mM MnCl2 , pH 7.0 at 25℃.)

3. Add the DNase I mixture (80 µl) directly to the RB column membrane, and incubate at 20–30℃ 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 15 s at full speed (10,000 x g, 13,000 rpm). Discard the flow-through.

Continue with the R-Wash Buffer wash step.

Recommended Step : DNA residue degradation

Procedure B (For Maxi Column)

1. *Add 2 ml Buffer R-W1 to the RB column. Close the lid gently, and centrifuge for 15 s 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 (RBC Cat. No. RN050) stock solution (see RN050 manual) to 130 µl Buffer RDD or DNase reaction buffer(not provided; 1MNacl, 20mM Tris-HCl, 10mM MnCl2 , pH 7.0 at 25°C.) 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 (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 140 µl DNase reaction buffer.*

(1MNacl, 20mM Tris-HCl, 10mM MnCl2 , pH 7.0 at 25°C.)

3. *Add the DNase I mixture (140 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 2 ml 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 R-Wash Buffer wash step.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