RNA Extraction Kit

User Guide

FOR RESEARCH USE ONLY

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1. Introduction

Quintech RNA Miniprep Kit provides an easy and fast method for isolating RNA from animal cells, bacteria, plant and fungi within 30 min. Only trace genomic DNA (gDNA) exists in the purified RNA, which can be eliminated by DNase I treatment when necessary. There is a DNA clearance step that could be used for gDNA extraction for downstream experiments.

Our proprietary RNA column and DNA clearance column result in a highly efficient and reversible binding of RNA, with proteins and other impurities removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

2. Kit Components & Safety Information

Catalog#	QT100-1301S SAMPLE	QT100-1302 50 Preps	QT100-1301 250 Preps
Preps	5	50	250
RNA Columns	5	50	250
DNA Clearance Columns	5	50	250
1.5 ml RNase-free microcentrifuge tubes	5	50	250
Buffer RLY+	3 ml	30 ml	150 ml
Buffer RW**	3 ml with ethanol added	20 ml	80 ml
RNAWash Buffer*	6.5 ml with ethanol added	24 ml	2 X 40 ml
DEPC Treated Water	1 ml	5ml	25 ml
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*96 ml (50 preps) or 160 ml (250 preps) 100% ethanol to each RNA Wash Buffer before use.

**30 ml (50 preps) or 120 ml (250 preps) 100% ethanol to each Buffer RW before use.

+Buffer RLY would precipitate below room temperature. It is critical to warm up the buffer at 50°C to disssolve the precipitate before use.

3. Storage

Quintech RNA Miniprep Kit should be stored dry at room temperature. Product performance and quality is maintained for kits stored up to 12 months at room temperature. For longer storage, kits can be stored at 2-8°C. Should any precipitate be observed in the buffers, redissolve at 37°C.

4. Supplies Needed

Materials to be supplied by user:
1.5 ml microcentrifuge tubes
95-100% ethanol
High speed microcentrifuge (13,000 rpm)
55-60°C waterbath

Materials to be prepared:
96 ml (50 preps) or 160 ml (250 preps) 100% ethanol to each RNA Wash Buffer before use.

Add 30 ml (50 preps) or 120 ml (250 preps) 100% ethanol to each Buffer RW before use.

Buffer RLY would precipitate below room temperature. It is critical to warm up the buffer at 50℃ to disssolve the precipitate before use.

Perform all steps including centrifugations at room temperature

5. Protocol for Extracting Total RNA from Plant Tissue and Filamentous Fungi

1. Weigh **50 mg** plant tissue (no more than 100 mg) or 3-4x10^6 cells in a 2 ml tube. Freeze the plant tissue in liquid nitrogen and grind using a rotor starter.

2. Transfer **5 volumes of (500 µl) Buffer RLY / β-mercaptoethanol** to the tube containing the plant tissue immediately. Grind using a rotor starter again.

Note: Ensure that β-mercaptoethanol has been added before use**.** 10 µl β-mercaptoethanol should be added in 500 µl RLY .

3. Transfer the cleared lysate to a DNA Clearance Column pre-inserted in a 2 ml collection tube. Centrifuge at 13,000 rpm for 2 min. Discard the DNA Clearance Column and save the flow-through.

Note: This step is for genomic DNA removal.

Optional: To obtain the gDNA, elution can be performed with 50 µl TE and centrifuge at 13,000 rpm for 1 min.

4. Add **1 volume of 100% ethanol** to the lysate (for example: **500 µl 100% ethanol** to **500 µl lysate**).

5. 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 place the column onto a new collection tube.

6. Add **400 µl Buffer RW** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.

Optional: To ensure complete removal of DNA, DNase I can be added for incubation on the column for 20 min at room temperature prior to the addition of the RNA Wash Buffer in step 7.

7. Add **500 µl RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.

Note: Ensure that ethanol has been added to RNA Wash Buffer before use.

8. Add another **500 µl RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube. Place the column, **with the lid open**, back to a new collection tube.

9. Centrifuge at 13,000 rpm for 5 min. Discard the flow-through.

Note: The residual ethanol will be removed more efficiently with the column lid opened during centrifugation.

10. Place the column to a RNase-free 1.5 ml tube, add **30-50 µl DEPC-treated ddH_2O** to the column, incubate at room temperature for 1 min, and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C. Reload the eluted RNA solution to the column and centrifuge at 13,000 rpm again.

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 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.

Note for gDNA applications: It is recommended that the eluted gDNA be used for downstream applications immediately due to the rapid autocatalysis.

6. Protocol for Extracting Total RNA from Cultured Cells

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

   Suspension cultured cells: Determine cell numbers and collect cells by centrifuging at 300 x g for 5 min. Remove all supernatant completely by aspiration and proceed to step 2.

   Adherent cultured cells: Determine cell numbers and aspirate the medium completely with a Pasteur pipette. Go to step 2 immediately by adding Buffer RLY.

   Note: Supernatant must be removed completely. Residual supernatant will inhibit cell lysis and thus affect the RNA yield.

2. **Suspension cells:** Flick the tube to loosen cell pellet and add **500 µl Buffer RLY.**

   Adherent cells: Add **500 µl Buffer RLY** directly into the dish. Use the pipette tip to mix and transfer the cell lysate to a 1.5 ml tube.

   Note: Determine the volume of Buffer RLY to be used and add 20 µl of β-mercaptoethanol per 1 ml Buffer RLY before use. Buffer RLY containing β-mercaptoethanol can be stored at room temperature for up to 1 month.

3. Homogenize the lysate by vortexing vigorously or repeated pipetting until the sample is uniformly homogenized. If the solution is clear, go to step 5, otherwise go to step 4.

4. Transfer the cleared lysate to a DNA Clearance Column pre-inserted in a 2 ml Collection Tube. Centrifuge at 13,000 rpm for 2 min. Discard the DNA Clearance Column (if gDNA not needed) and save the flow-through.

   Note：This step is for genomic DNA removal.

   Optional: To obtain gDNA, elution can be performed with 50 µl TE. Centrifuge at 13,000 rpm for 1 min.

5. Add **1 volume 70% ethanol** into the lysate **(for example: 500 µl 70% ethanol for 500 µl lysate)** and pipet 5 times to mix the solution. Vortex briefly to remove precipitation.

6. Transfer the solution to a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column into a new collection tube.

7. Add **500 µl Buffer RW** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.

   Optional: To ensure complete removal of DNA, DNase I can be added for incubation on the column for 20 min at room temperature prior to the addition of the RNA Wash Buffer in step 8.

8. Add **500 µl RNA Wash Buffer** *(Add ethanol before use)* to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.

9. Add another **600 µl RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, **with the lid open**, into a new collection tube. Centrifuge the column at 12,000 rpm for 5 min.

   Note: The residual ethanol will be removed more efficiently with the lid of the column open.

10. Transfer the column to a RNase-free 1.5 ml tube and add **30-50 µl DEPC-treated ddH2O** to the center of the column, incubate at room temperature for 1 min. Centrifuge at 13,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20 °C.

7. Protocol for Extracting Total RNA from Gram-Positive (B.subtilis) or Gram-Negative (E.coli) Bacteria

1. Prepare a lysozyme stock solution at 3 mg /ml or 0.4 mg /ml with Elution Buffer or TE Buffer and aliquot into adequate portions. Store each aliquot at -20℃and thaw before use.

2. Harvest no more than **3 ml** culture (< $5x10^8$) by centrifugation at 10,000 rpm for 2 min.

3. Carefully remove the supernatant as much as possible.

4. Resuspend the pellet in **100 µl** freshly prepared **TE Buffer (10mM,Tris-HCl, pH 8.0;1mM EDTA, pH 8.0) or Elution Buffer (10mM Tris-HCl, PH 8.5)** containing lysozyme. (**Use 3 mg lysozyme per 1 ml TE Buffer for gram-positive bacteria and 1 mg /ml lysozyme for gram-negative bacteria**). Mix by tapping gently.

5. Incubate the resuspended pellet at room temperature for 5-10 min for gram-positive bacteria or 2 min for gram-negative bacteria.

6. Add **400 µl Buffer RLY**. Mix gently. Transfer the clear lysate to a DNA Clearance Column pre-inserted into a 2 ml collection tube. Centrifuge at 13,000 rpm for 2 min. Discard the DNA Clearance Column and save the flow-through.

   Note: This step is for genomic DNA removal.

   Optional: To obtain gDNA, elution can be performed with 50 µl TE and centrifuge at 13,000 rpm for 1 min.

   Note: Ensure that β-mercaptoethanol has been added before use.

7. Transfer flow-through to a new RNase-free tube. Add **0.7 volume of 100% ethanol** to the lysate (For example: 350 µl 100% ethanol for 500 µl lysate).

8. Transfer the solution into the binding column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and place the column into a new collection tube.

   Optional: To ensure complete removal of DNA, DNase I can be added for incubation on the column for 20 min at room temperature prior to the addition of the RNA Wash Buffer in step 9.

9. Add **500 µl Buffer RW** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.

10. Add **500 ml RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through.

    Note: Ensure that ethanol has been added to RNA Wash Buffer before use.

11. Add another **600 µl RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column into a new collection tube.

12. Centrifuge the column at 13,000 rpm, **with the lid opened,** for another 5 min.

    Note: It is critical to remove residual ethanol for optimal elution.

13. Place the column into a RNase-free 1.5 ml tube. Add **30-50 µl DEPC-treated ddH_2O** to the column and incubate at room temperature for 1 min. Centrifuge at 13,000 rpm for 2 min. The RNA is in the flow-through liquid. Store the RNA solution at -20 ℃.

2. **Note:** It is highly recommended that RNA quality be determined before any downstream applications are carried out. Quality of RNA can be assessed by employing denatured agarose gel electrophoresis, followed by 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.

   Note for gDNA applications: It is recommended that the eluted gDNA be used for downstream applications immediately due to the rapid autocatalysis.

8. Troubleshooting Guide

Problem	Possible Reason	Recommended Solution
Low A_{260}/A_{280} ratios	Protein contamination	Do a Phenol: Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low yield	RNA in sample is degraded	Freeze samples immediately in liquid nitrogen and store at -70°C after collection.
Low yield	The binding capacity of the membrane in the spin column was exceeded	Excessive use of tissue sample exceeding the binding capacity of spin column will cause decrease in total RNA yield.
Low yield	Ethanol not added to buffer	Add ethanol to the RNA Wash Buffer and Buffer RW before purification.
Genomic DNA contamination	Excess 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 $1\text{-}2x10^6$ or increase buffer volume with multiple loadings to column.

13. Ordering Information

Catalog #	Description
QT100-1301	Quintech RNA Extraction Kit, 250 preps Includes 250 RNA columns, 250 collection tubes, 250 sample tubes, reagents and user guide
QT100-1302	Quintech RNA Extraction Kit, 50 preps Includes 50 RNA columns, 50 collection tubes, 50 sample tubes, reagents and user guide

Kit Components

QT100-8103C	RNA Mini Columns, 250 preps
QT100-8104C	RNA Mini Columns, 50 preps
QT100-8105C	DNA Clearance columns, 250 preps
QT100-8106C	DNA Clearance columns, 50 preps
QT100-8107C	1.5ml RNAse-free tubes, 250 preps
QT100-8108C	1.5ml RNAse-free tubes, 50 preps
QT100-8219RLY	Buffer RLY, 150 ml, 250 preps
QT100-8220RLY	Buffer RLY, 30 ml, 50 preps
QT100-8221RW	Buffer RW, 80 ml, 250 preps
QT100-8222RW	Buffer RW, 20 ml, 50 preps
QT100-8223RNA	RNA Wash Buffer, 40 ml, 2, 250 preps
QT100-8224RNA	RNA Wash Buffer, 24 ml, 50 preps
QT100-8225H2O	DEPC-Treated Water, 25ml, 250 preps
QT100-8226H2O	DEPC-Treated Water, 5ml, 50 preps

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Simplifying Sample Purification

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Version 2