Cyrusbioscience

Total RNA Mini Kit Sample

Sample: 500 ul Whole Blood, 10^6 Cultured Cells, 10^8 Bacteria (Gram +/-), 10^7 Yeast, 10 mg Tissue

Yield: Up to 50 ug

Format: Spin Columns

Operation: Centrifuge or Vacuum

Operation Time: 20-30 Minutes

Cat. Number:

Storage/Stability:

Store at room temperature 15°C~25°C

Features:

Complete removal of all contaminants for reliable downstream applications. No phenol, chloroform or alcohol. Rapid and simple procedure.

Description:

Total RNA Mini Kit is special designed for purification of total RNA from cultured cells, fresh human whole blood, bacteria and Tissue. The method use detergents and a chaotropic salt to lyse cell and inactivate RNase, than RNA in chaotropic salt is bonded to glass fiber matrix of column. After washing off the contaminants, the purified RNA is eluted by RNase-free water. The entire procedure can be completed in 20 minutes and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

Applications:

Purified RNA is ready for direct use in RT-PCR, Real-Time RT-PCR, Northern Blotting, Primer Extension, RNase Protection Assays, mRNA Selection, cDNA Synthesis.

Quality Control:

The quality of Total RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 300 ul of fresh human whole blood. More than 1 ug of total RNA was quantified with a spectrophotometer and checked by formaldhyde agarose gel analysis. Finally, RT-PCR was used to ensure the quality of total RNA.

Kit Contents:

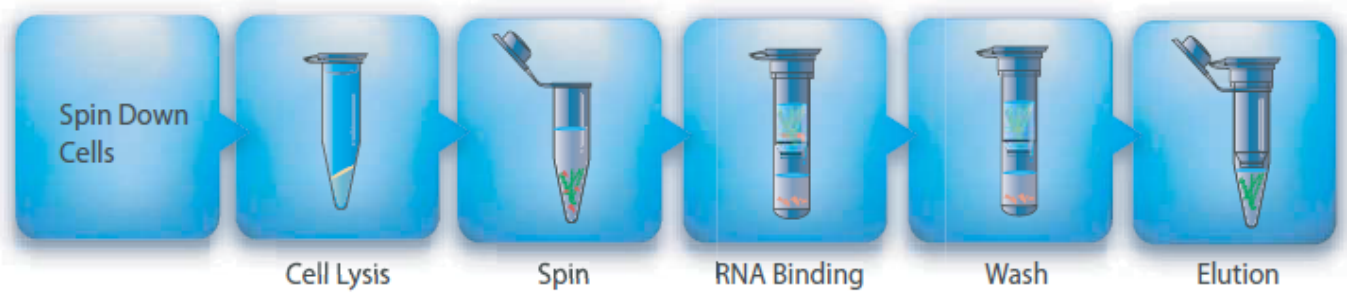
RBC Lysis Buffer	10 ml
RT Buffer	2 ml
RC Buffer	2 ml
70% Ethanol	2 ml*
W1 Buffer	4 ml
W2 Buffer (concentrated)	5 ml**

RNase free water	1 ml
RNA Column	4 pcs
Collection Tube	4 pcs

* Ethanol(95-100%) has been added in the tube.

** Ethanol(95-100%) has been added in W2 Buffer.

Blood / Cultured Cell Protocol:



Additional Requirements:

ß - Mercaptoethanol

DNase I stock: Dissolve 2,500units DNase I in 0.55 ml RNase free water (4.5 U/ ul) and store at -20°C.

DNase Incubation Buffer: (1 M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C)

Use fresh human blood :

1. Collect fresh human blood in anticoagulant-treated collection tubes.
2. Add 1 ml RBC Lysis Buffer to a sterile 1.5 ml reaction tube.
3. Add 500 ul human whole blood and mix by inversion. Do not vortex.
4. Incubate the tube for 10 minutes at room temperature. During incubation, invert the tube every 2-3 minutes..
5. Centrifuge for 3 minutes at 2500 rpm (500 × g) in a microcentrifuge.
6. Discard the supernatant, add 1 ml RBC Lysis Buffer to the tube and mix by inversion. Do not vortex.
7. Incubate the tube for 3 minutes at room temperature.
8. Centrifuge for 3 minutes at 2500 rpm (500 × g) in a microcentrifuge.
9. Discard the supernatant and resuspend the cells in 100 ul of RBC Lysis Buffer by flicking the tube and follow the Cell Lysis step.

Use Cultured animal cells :

1. If using adherent cells, trypsinize the cells before harvesting.
2. Transfer 10 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 20 seconds at 6,000 × g (8,000 rpm).
3. Discard the supernatant and resuspend the cells in 100 ul PBS or RBC Lysis Buffer.

Cell Lysis

7. Add 400 ul RC Buffer and 4 ul ß-Mercaptoethanol to the sample from RBC Lysis Step, mix by vortexing.

8. Incubate at room temperature for 5 minutes.

9. Centrifuge for 1 minutes at full 13,000 rpm.

10. Transfer the clarified supernatant to a new microcentrifuge tube (not provided).

RNA Binding

11. Place a RNA Column in a Collection tube.

12. Add 400 ul 70% ethanol to the sample lysate from Cell Lysis Step and mix immediately by pipetting.

13. Apply 750 ul of ethanol-added mixture from previous step to the RNA Column.

14. Centrifuge at 13,000 rpm for 1 minute.

15. Discard the flow-through and place the RNA Column back in the Collection Tube, apply the rest mixture to the same Column.

16. Centrifuge at 13,000 rpm for 3 minutes.

17. Discard the flow-through and place the RNA Column back in the Collection Tube.

Optional Step: DNA residue degradation

a. Apply 400 ul W1 Buffer into the column.

b. Centrifuge at 13,000 rpm for 1 minute.

c. Discard the flow-through and place the RNA Column back in the Collection Tube.

d. Add 100 ul DNase I buffer (45 Units, 10 ul DNase I + 90 ul DNase Incubation Buffer) to the center of the RNA Column matrix.

e. Stand for 10 minutes at room temperature.

f. Go to Wash Step.

Wash

18. Apply 400 ul W1 Buffer into the column.

19. Centrifuge at 13,000 rpm for 1 minute.

20. Discard the flow-through and place the RNA Column back in the Collection Tube.

21. Add 600 ul W2 Buffer (ethanol added) into the column.

22. Centrifuge at 13,000 rpm for 1 minute.

23. Discard the flow-through and place the RNA Column back in the Collection Tube.

24. Centrifuge at 13,000 rpm for 3 minutes to dry the column matrix.

RNA Elution

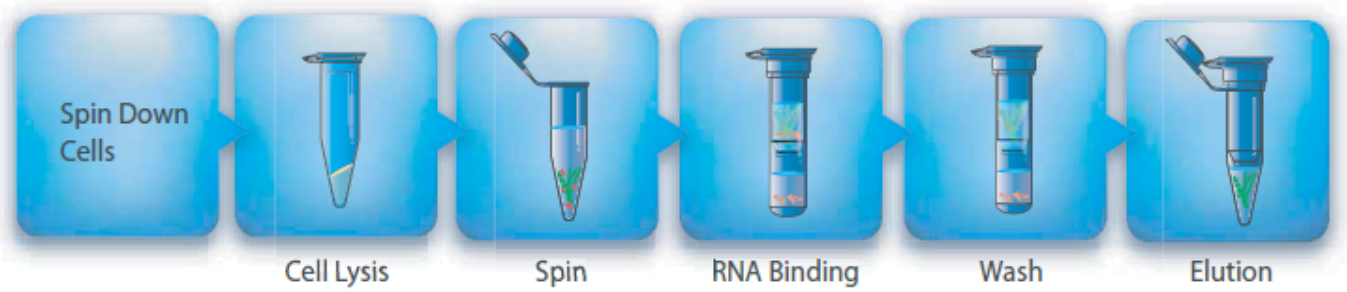
25. Place dried RNA Column in a clean microcentrifuge tube (RNase-free, not provided).

26. Add 50 ul RNase-free water (use preheated to 70°C RNase-free water can increase the yield) into the center of the column matrix.

27. Stand for 3 minutes until water has been absorbed by the matrix.

28. Centrifuge at full speed for 2 minutes to elute purified RNA.

Bacterial Protocol:



Additional Requirements:

ß - Mercaptoethanol

DNase I stock: Dissolve 2,500units DNase I in 0.55 ml RNase free water (4.5 U/ ul) and store at -20°C.

DNase Incubation Buffer: (1 M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C)

Lysozyme Buffer (30 mg/ml lysoyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0)

Cell Harvesting

For Gram-negative bacteria :

1. Transfer bacterial culture (< 10^8) to a microcentrifuge tube (not provided).

2. Centrifuge for 3 minutes at 6,000 rpm in a microcentrifuge and discard the supernatant and then vortexing the cell pellet

for 30 seconds.

3. Add 200 ul RT Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.

4. Incubate at room temperature for 5 minutes.

For Gram-positive bacteria :

1. Transfer bacterial culture (< 10^8) to a microcentrifuge tube (not provided).

2. Centrifuge for 3 minutes at 6,000 rpm in a microcentrifuge and discard the supernatant.

3. Add 200 ul Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.

4. Incubate at room temperature for 2-4 hours. During incubation, vortex the tube several times.

Cell Lysis

5. Add 300 ul RC Buffer to the sample lysate from Cell Harvesting Step, mix by vortexing.

6. Incubate at room temperature for 5 minutes.

7. Centrifuge at 13,000 rpm for 1 minutes.

8. Transfer the supernatant to a new microcentrifuge tube.

RNA Binding

9. Place a RNA Column in a Collection tube.

10. Add 300 ul 70% ethanol to the sample lysate from Cell Lysis Step and mix immediately by pipetting.

11. Apply 750 ul of ethanol-added mixture from previous step to the RNA Column.

12. Centrifuge at 13,000 rpm for 1 minute.

13. Discard the flow-through and place the RNA Column back in the Collection Tube, apply the rest mixture to the same Column.

14. Centrifuge at 13,000 rpm for 3 minutes.

15. Discard the flow-through and place the RNA Column back in the Collection Tube.

Optional Step: DNA residue degradation

a. Apply 400 ul W1 Buffer into the column.

b. Centrifuge at 13,000 rpm for 1 minute.

c. Discard the flow-through and place the RNA Column back in the Collection Tube.

d. Add 100 ul DNase I buffer (45 Units, 10 ul DNase I + 90 ul DNase Incubation Buffer) to the center of the RNA Column matrix.

e. Stand for 10 minutes at room temperature.

f. Go to Wash Step.

Wash

16. Apply 400 ul W1 Buffer into the column.

17. Centrifuge at 13,000 rpm for 1 minute.

18. Discard the flow-through and place the RNA Column back in the Collection Tube.

19. Add 600 ul W2 Buffer (ethanol added) into the column.

20. Centrifuge at 13,000 rpm for 1 minute.

21. Discard the flow-through and place the RNA Column back in the Collection Tube.

22. Centrifuge at 13,000 rpm for 3 minutes to dry the column matrix.

RNA Elution

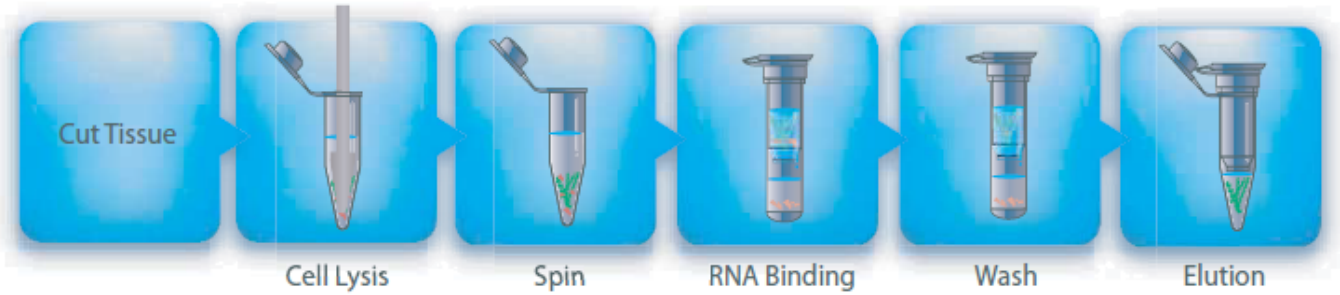
23. Place dried RNA Column in a clean microcentrifuge tube (RNase-free, not provided).

24. Add 50 ul RNase-free water (use preheated to 70°C RNase-free water can increase the yield) into the center of the column matrix.

25. Stand for 3 minutes until water has been absorbed by the matrix.

26. Centrifuge at full speed for 2 minutes to elute purified RNA.

Tissue Protocol:



Additional Requirements:

ß - Mercaptoethanol

70% Ethanol (RNase free)

DNase I stock: Dissolve 2,500units DNase I in 0.55 ml RNase free water (4.5 U/ ul) and store at -20°C.

DNase Incubation Buffer: (1 M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C)

Cell Lysis

1. Cut off 10 mg (do not over 20 mg) of fresh or frozen animal tissue and transfer it into a microcentrifuge tube(not provided).
2. Add 400 ul RC Buffer and 4 ul ß-Mercaptoethanol into the tube and use Micropestle (not provided) to ground the tissue a few times into small pieces and then vortex the tube for 5 seconds.
3. Incubate at room temperature for 5 minutes to lysis sample.
4. Vortex the tube for 5 seconds.
5. Centrifuge for 3 minutes at 13,000 rpm.
6. Transfer the clarified supernatant to a new microcentrifuge tube (not provided).

RNA Binding

7. Place a RNA Column in a Collection tube.
8. Add 400 ul 70% ethanol to the sample lysate from Cell Lysis Step and mix immediately by pipetting.
9. Apply ethanol-added mixture from previous step to the RNA Column.
10. Centrifuge at 13,000 rpm for 3 minutes.
11. Discard the flow-through and place the RNA Column back in the Collection Tube.

Optional Step: DNA residue degradation

a. Apply 400 ul W1 Buffer into the column.

b. Centrifuge at 13,000 rpm for 1 minute.

c. Discard the flow-through and place the RNA Column back in the Collection Tube.

d. Add 100 ul DNase I buffer (45 Units, 10 ul DNase I + 90 ul DNase Incubation Buffer) to the center of the RNA Column matrix.

e. Stand for 10 minutes at room temperature.

f. Go to Wash Step.

Wash

12. Apply 400 ul W1 Buffer into the column.

13. Centrifuge at 13,000 rpm for 1 minute.

14. Discard the flow-through and place the RNA Column back in the Collection Tube.

15. Add 600 ul W2 Buffer (ethanol added) into the column.

16. Centrifuge at 13,000 rpm for 1 minute.

17. Discard the flow-through and place the RNA Column back in the Collection Tube.

18. Centrifuge at 13,000 rpm for 3 minutes to dry the column matrix.

RNA Elution

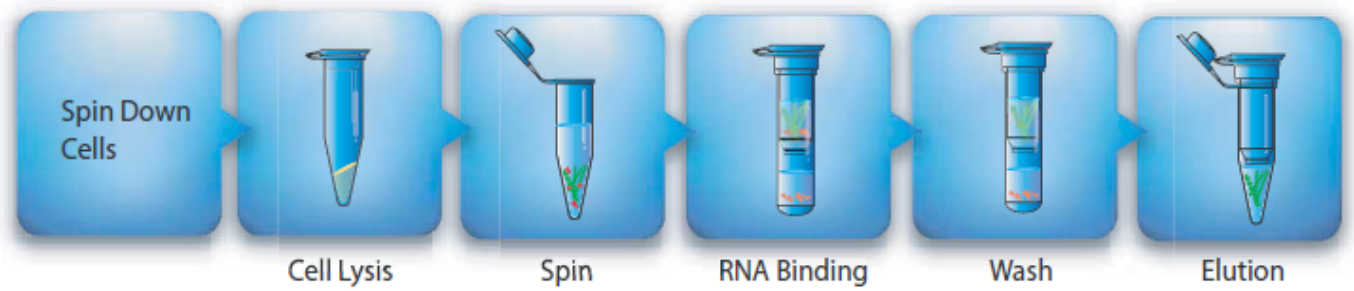
19. Place dried RNA Column in a clean microcentrifuge tube (RNase-free, not provided).

20. Add 50 ul RNase-free water (use preheated to 70°C RNase-free water can increase the yield) into the center of the column matrix.

21. Stand for 3 minutes until water has been absorbed by the matrix.

22. Centrifuge at full speed for 2 minutes to elute purified RNA.

Yeast Protocol:



Additional Requirements:

ß - Mercaptoethanol

DNase I stock: Dissolve 2,500units DNase I in 0.55 ml RNase free water (4.5 U/ ul) and store at -20°C.

DNase Incubation Buffer: (1 M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C)

Sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1M Tris-Cl pH 7.5; 35 mM mercaptoethanol)

Lyticase or Zymolase

Cell Harvesting

1. Harvest the cells (2 x 10^7 cells) in a 15 ml centrifuge tube by centrifuging at1000 x g for 5 minutes.

2. Decant the supernatant, and carefully remove any remaining media by aspiration.

(Incomplete removal of medium will affect digestion of the cell wall).

3. Resuspend the cells in 2 ml freshly sorbitol Buffer containing lyticase or zymolase.

(final concentration of 100 U per 2 x 10^7 cells, Depending on the yeast strain and enzyme used, the incubation time,

enzyme concentration, and composition of Buffer may vary. Please follow the guidelines of the enzyme supplier).

4. Incubate for 30 minutes at 30°C with gentle shaking to generate spheroplasts. Spheroplasts must be handled gently.

5. Centrifuge for 5 minutes at 300 × g to pellet the spheroplasts. Carefully remove and discard the supernatant.

Note: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the membrane. Both effects may reduce RNA yield.

Cell Lysis

6. Add 400 ul RC Buffer to the sample lysate from Cell Harvesting Step, mix by vortexing.

7. Incubate at room temperature for 5 minutes.

8. Centrifuge at 13,000 rpm for 1 minutes.

9. Transfer the supernatant to a new microcentrifuge tube.

RNA Binding

10. Place a RNA Column in a Collection tube.

11. Add 400 ul 70% ethanol to the sample lysate from Cell Lysis Step and mix immediately by pipetting.

12. Apply 750 ul of ethanol-added mixture from previous step to the RNA Column.

13. Centrifuge at 13,000 rpm for 1 minute.

14. Discard the flow-through and place the RNA Column back in the Collection Tube, apply the rest mixture to the same Column.

15. Centrifuge at 13,000 rpm for 3 minutes.

16. Discard the flow-through and place the RNA Column back in the Collection Tube.

Optional Step: DNA residue degradation

a. Apply 400 ul W1 Buffer into the column.

b. Centrifuge at 13,000 rpm for 1 minute.

c. Discard the flow-through and place the RNA Column back in the Collection Tube.

d. Add 100 ul DNase I buffer (45 Units, 10 ul DNase I + 90 ul DNase Incubation Buffer) to the center of the RNA Column matrix.

e. Stand for 10 minutes at room temperature.

f. Go to Wash Step.

Wash

17. Apply 400 ul W1 Buffer into the column.

18. Centrifuge at 13,000 rpm for 1 minute.

19. Discard the flow-through and place the RNA Column back in the Collection Tube.

20. Add 600 ul W2 Buffer (ethanol added) into the column.

21. Centrifuge at 13,000 rpm for 1 minute.

22. Discard the flow-through and place the RNA Column back in the Collection Tube.

23. Centrifuge at 13,000 rpm for 3 minutes to dry the column matrix.

RNA Elution

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

25. Add 50 ul RNase-free water (use preheated to 70°C RNase-free water can increase the yield) into the center of the column matrix.

26. Stand for 3 minutes until water has been absorbed by the matrix.

27. Centrifuge at full speed for 2 minutes to elute purified RNA.

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

Note:(1) RC Buffer contains chaotropic salt is harmful and irritant agent. (2) Use sterile, RNase-free pipet tips and microcentrifuge tube. *Wear a lab coat and disposable gloves to prevent RNase contamination.