Cyrusbioscience

microRNA Mini Kit Sample

Sample: 500 ul Whole Blood, 10^6 Cultured Cells, 10^8 Bacteria (Gram +/-), $5x10^6$ Yeast, 10 mg Tissue

Yield: Up to 20 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:

microRNA Mini Kit provides a rapid method for the isolation and purification of small RNA molecules (< 200 nt) from cultured animal cells, tissue samples, bacterial cells, plants and blood. These small RNAs include regulatory RNA molecules such as microRNA(miRNA) and short interfering RNA (siRNA), as well as tRNA and 5S rRNA. Small RNA molecules are often studied due to their ability to regulate gene expression. miRNAs and siRNAs are typically 20-25 nucleotides long, and regulate gene expression by binding to mRNA molecules and affecting their stablility or translation. The small RNA molecules isolated using microRNA Mini Kit can be use in various downstream applications relating to gene regulation and functional analysis, including RT-PCR, northern blotting and microarray analysis.

Applications:

Purified RNA is ready for direct use in RT-PCR, Real-Time RT-PCR, Northern Blotting, Microarray Analysis.

Quality Control:

The quality of microRNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of microRNA from 500 ul of fresh human whole blood. More than 1 ug of microRNA 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:

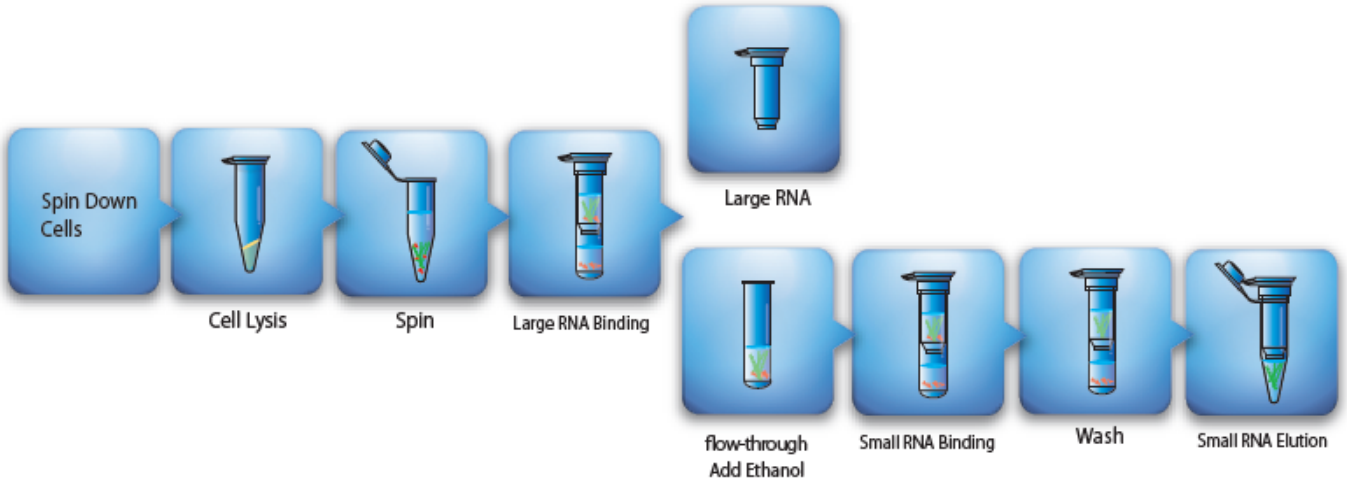
RBC Lysis Buffer	10 ml
RT Buffer	2 ml
RC Buffer	2 ml
70% Ethanol*	2 ml

W2 Buffer **	5 ml
RNase free water	1 ml
RNA Column(Yellow Ring)	4 pcs
miRNA Column (Blue Ring)	4 pcs
Collection Tube	8 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

Ethanol (96-100%)

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. Follow the Cell Lysis step.

Cell Lysis

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

11. Incubate at room temperature for 5 minutes.

12. Vortex for 5 seconds and then centrifuge for 1 minutes at full 13,000 rpm.

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

Large RNA Binding

14. Place a RNA Column in a Collection tube.

15. Add 400 ul 70% ethanol (RNase-free) to the sample lysate from Cell Lysis Step and mix immediately by vortexing.

16. Apply ethanol-added mixture from previous step to the RNA Column.

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

18. Transfer the flow-through to a new microcentrifuge tube(not provided).

19. Add 600 ul ethanol (96-100%) to the flow-through and mix by vortexing.

Small RNA Binding

20. Place a miRNA Column in a new Collection tube.

21. Apply 700 ul of ethanol-added mixture from step 19 to the miRNA Column, centrifuge at 13,000 rpm for 1 minute.

22. Discard the flow-through and apply the rest mixture to the same column, centrifuge at 13,000 rpm for 1 minute.

23. Discard the flow-through.

Wash

24. Apply 600 ul W2 Buffer (ethanol added) into the miRNA Column.

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

26. Discard the flow-through and place the miRNA Column back in the Collection Tube.

27. Add 600 ul W2 Buffer (ethanol added) into the miRNA Column.

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

29. Discard the flow-through and place the miRNA Column back in the Collection Tube.

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

Small RNA Elution

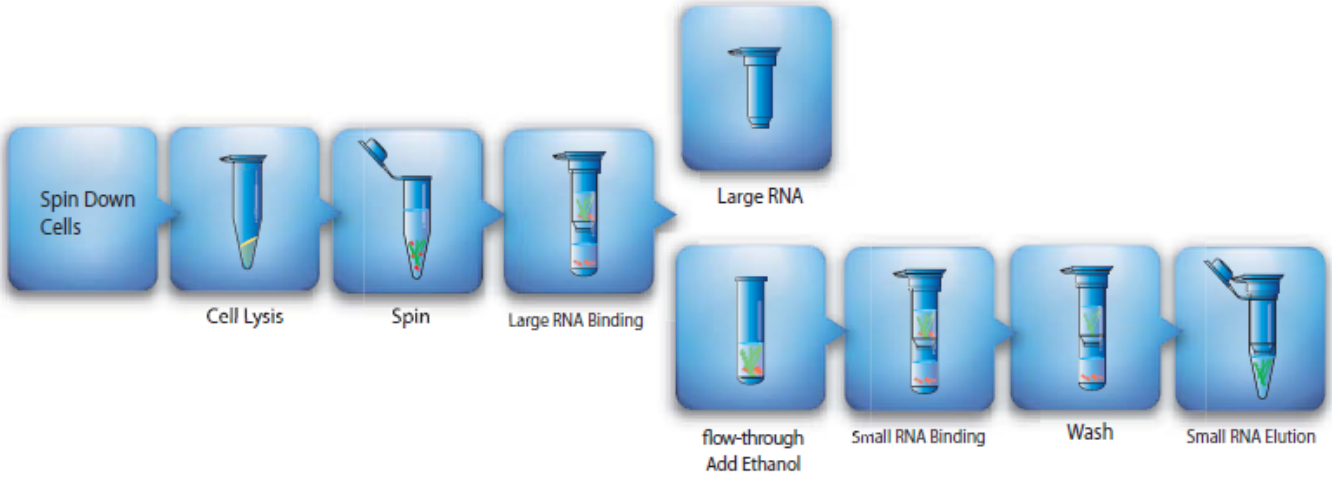
31. Place dried miRNA Column in a clean microcentrifuge tube (RNase-free, not provided).

32. 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.

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

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

Bacterial Protocol:



Additional Requirements:

ß - Mercaptoethanol

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

Ethanol (96-100%)

Cell Harvesting

For Gram-negative bacteria :

1. Transfer bacterial culture (< 10^9) 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 (< 10^9) 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 20 minutes. During incubation, invert the tube every 2-3 minutes.

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.

Large RNA Binding

9. Place a RNA Column in a Collection tube.

10. Add 300 ul 70% ethanol (RNase-free) to the sample lysate from Cell Lysis Step and mix immediately by vortexing.

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

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

13. Transfer the flow-through to a new microcentrifuge tube(not provided).

14. Add 600 ul ethanol (96-100%) to the flow-through and mix by vortexing.

Small RNA Binding

15. Place a miRNA Column in a new Collection tube.

16. Apply 700 ul of ethanol-added mixture from step 14 to the miRNA Column, centrifuge at 13,000 rpm for 1 minute.

17. Discard the flow-through and apply the rest mixture to the same column, centrifuge at 13,000 rpm for 1 minute.

18. Discard the flow-through.

Wash

19. Apply 600 ul W2 Buffer (ethanol added) into the miRNA Column.

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

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

22. Add 600 ul W2 Buffer (ethanol added) into the miRNA Column.

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

24. Discard the flow-through and place the miRNA Column back in the Collection Tube.

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

Small RNA Elution

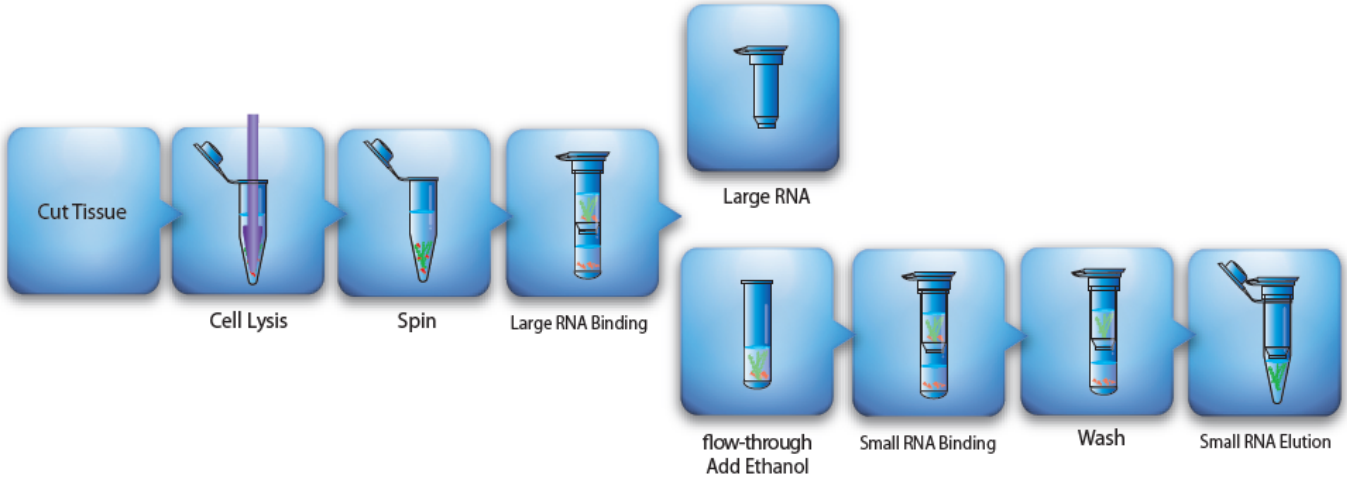
26. Place dried miRNA Column in a clean microcentrifuge tube (RNase-free, not provided).

27. 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.

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

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

Tissue Protocol:



Additional Requirements:

ß - Mercaptoethanol

Ethanol (96-100%)

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 10 seconds.
5. Centrifuge for 3 minutes at 13,000 rpm.
6. Transfer the clarified supernatant to a new microcentrifuge tube (not provided).

Large RNA Binding

7. Place a RNA Column in a Collection tube.
8. Add 400 ul 70% ethanol (RNase-free) to the sample lysate from Cell Lysis Step and mix immediately by vortexing.
9. Apply ethanol-added mixture from previous step to the RNA Column.
10. Centrifuge at 13,000 rpm for 1 minute.
11. Transfer the flow-through to a new microcentrifuge tube(not provided).
12. Add 600 ul ethanol (96-100%) to the flow-through and mix by vortexing.

Small RNA Binding

13. Place a miRNA Column in a new Collection tube.
14. Apply 700 ul of ethanol-added mixture from step 12 to the miRNA Column, centrifuge at 13,000 rpm for 1 minute.
15. Discard the flow-through and apply the rest mixture to the same column, centrifuge at 13,000 rpm for 1 minute.
16. Discard the flow-through.

Wash

17. Apply 600 ul W2 Buffer (ethanol added) into the miRNA Column.
18. Centrifuge at 13,000 rpm for 1 minute.
19. Discard the flow-through and place the miRNA Column back in the Collection Tube.
20. Add 600 ul W2 Buffer (ethanol added) into the miRNA Column.
21. Centrifuge at 13,000 rpm for 1 minute.
22. Discard the flow-through and place the miRNA Column back in the Collection Tube.
23. Centrifuge at 13,000 rpm for 3 minutes to dry the column matrix.

Small RNA Elution

24. Place dried miRNA 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.