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miRNA Extraction Kit Protocol Book

Tissue/Cultured Cells

Reliable miRNA Purification from Tissue and Cultured Cells

Cat.No. **YMR50S/YMR50/YMR100**

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miRNA Extraction Kit

Tissue/Cultured Cells



Cat.No. YMR50S

4 preps / kit
M1 Buffer: 1 ml
M2 Buffer: 1.5 ml
Wash Buffer: 250 μ l *
Release Buffer: 1 ml
RNA Column: 8 pcs
2 ml Collection Tube: 8 pcs
All components are RNase-Free.

Cat.No. YMR50

50 preps / kit
M1 Buffer: 12.5 ml
M2 Buffer: 1.5 ml
Wash Buffer: 12.5 ml **
Release Buffer: 6 ml
RNA Column: 100 pcs
2 ml Collection Tube: 100 pcs
All components are RNase-Free.

Cat.No. YMR100

100 preps / kit
M1 Buffer: 25 ml
M2 Buffer: 3 ml
Wash Buffer: 12.5 ml **
Release Buffer: 6 ml
RNA Column: 200 pcs
2 ml Collection Tube: 200 pcs
All components are RNase-Free.

Sample: 100 mg Tissue, 1x10⁶ Cultured Cells
Purity: miRNA smaller than 100 nt can be purified
Format: Spin Columns
Operation: Centrifuge or Vacuum
Operation Time: 30 Minutes

- * Add 1 ml Ethanol (96-100%) to 250 μ l of Wash Buffer prior to initial use.
- ** Add 50 ml Ethanol (96-100%) to 12.5 ml of Wash Buffer prior to initial use.

miRNA Extraction Kit (Tissue/Cultured Cells)

Description

miRNA Extraction Kit is especially designed for purification of microRNAs (miRNAs) and other small cellular RNAs from tissue or cultured cells. Purification of miRNA allows research into biological significant pathways for gene regulation. For some sensitive downstream applications, an miRNA-enriched fraction without larger RNA are required. Therefore, miRNA Extraction Kit is specifically designed for effective purification of small RNA with minimal contamination from large RNA molecules or genomic DNA. The entire procedure can be completed in 30 minutes.

Features

Efficient enrichment of miRNA.
High-purity miRNA suitable for all downstream applications.
Effective purification of miRNA from a wide range of tissue and cultured cells.

Applications

Purified miRNA is ready for direct use in Genomic Regulation Research, qPCR, Microarray, Cell Expression and Transfection with siRNA.

Quality Control

The quality of miRNA Extraction Kits(Tissue/Cultured Cells) are tested on a lot-to-lot basis.

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

Note:(1) For research use only. Not for use in diagnostic or therapeutic procedures. (2) During operation, always wear a lab coat, disposable gloves, and protective goggles.

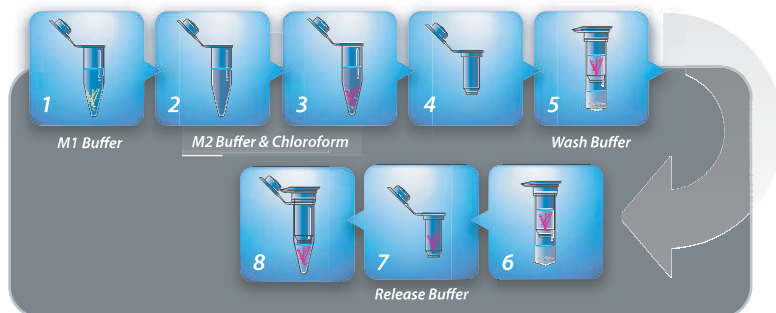
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miRNA Extraction Kit (Tissue/Cultured Cells)

Protocol

Additional requirements:

- * 96% ~ 100% Ethanol.
- * Sterile, RNase-free pipette tips and microcentrifuge tubes.
- * Chloroform.
- * ddH₂O Saturated Phenol.
- * Water Bath or Dry Bath or Microwave Oven.



Tissue Dissociation

1. Cut off up to 100 mg of animal tissue (or 1x10⁶ Cultured Cells pellet) and transfer the pellet into a microcentrifuge tube (not provided).
2. Add 200 μ l M1 Buffer into the tube and vigorously mixing by vortexing.
3. Incubate at room temperature for 10 minutes.

Lysis

4. Add 20 μ l M2 Buffer, 180 μ l ddH₂O Saturated Phenol and 40 μ l chloroform into the tube, vortex vigorously for 2 minutes.
5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the supernatant into a clean microcentrifuge(not provided).
6. Preheat required Release Buffer (50 μ l per sample) in a 65°C water bath (For Step 16 RNA Elution).

miRNA Binding

7. Add ethanol (96-100%) to 35% volume (ex. add 108 μ l of ethanol to 200 μ l of supernatant). Mix well by vortexing.
8. Transfer above mixture to the RNA Column in a 2 ml Collection Tube. Incubate at room temperature for 1 minute.
9. Centrifuge at 12,000 rpm for 30 seconds. Transfer the filtrate to a new 1.5ml microcentrifuge tube(RNase-free).
10. Add ethanol(96-100%) to 70% volume(ex. add 676 μ l of ethanol to 290 μ l of filtrate). Mix well by vortexing.
11. Transfer above mixture to another new RNA Column in a new 2 ml Collection Tube. Incubate at room temperature for 1 minute.
12. Centrifuge at 12,000 rpm for 30 seconds.

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3

Wash

13. Add 200 µl of ethanol-added Wash Buffer into the RNA Column. Incubate at room temperature for 1 minute.
14. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.

RNA Elution

15. Put the RNA Column into a clean 1.5 ml microcentrifuge tube (not provided).
16. Add 50 µl of preheated Release Buffer into the center of the column matrix.
17. Keep the 1.5 ml microcentrifuge tube stand at room temperature for 3 minutes until Release Buffer is absorbed by the matrix.
18. Centrifuge at 12,000 rpm for 3 minutes to elute purified miRNA.
19. Utilize 0.2 volume of purified miRNA to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its quality.

The purified miRNA can be further concentrated by a standard ethanol precipitation procedure and then re-dissolved in a small volume ddH₂O or TE buffer, pH 8.0).
The majority of RNA visible on the gel should be <100 nt in size, with the major bands corresponding to tRNAs. The 5S and 5.8S rRNA species may also be visible. These tRNA and small rRNA bands should be clear and distinct. miRNA (21-22 nt) are typically not visible on the gel image.

Notes

Solutions for Transformation, Cloning, Genomics and Proteomics: www.real-biotech.com

Ordering Information

	Cat.No.	Size	Items	Contents	
Total RNA	Blood & Bacteria	YRB50	S	Total RNA Extraction Kit [Mini] (Blood / Bacteria/Cultured Cell)	50 preps/kit, (RBC Lysis, RB, RT, Wash, W1) Buffer, RNase-free Water, etc.
		YRB100	S	Total RNA Extraction Kit [Mini] (Blood / Bacteria/Cultured Cell)	100 preps/kit, (RBC Lysis, RB, RT, Wash, W1) Buffer, RNase-free Water, etc.
		YRTM10	L	Total RNA Extraction Kit [Maxi] (Blood / Bacteria/Cultured Cell)	10 preps/kit, (RBC Lysis, RB, RT, Wash, W1) Buffer, RNase-free Water, etc.
	Tissue	YRT50	S	Total RNA Extraction Kit [Mini] (Tissue)	50 preps/kit, (RB, Wash, W1) Buffer, RNase-free Water, etc.
		YRT100	S	Total RNA Extraction Kit [Mini] (Tissue)	100 preps/kit, (RB, Wash, W1) Buffer, RNase-free Water, etc.
		YRT300	S	Total RNA Extraction Kit [Mini] (Tissue)	300 preps/kit, (RB, Wash, W1) Buffer, RNase-free Water, etc.
		YRTM10	L	Total RNA Extraction Kit [Maxi] (Tissue)	10 preps/kit, (RBC Lysis, RB, RT, Wash, W1) Buffer, RNase-free Water, etc.
	Plant	YRP50	S	Total RNA Extraction Kit [Mini] (Plant)	50 preps/kit, (RB, PRB, Wash, W1) Buffer, RNase-free Water, etc.
		YRP100	S	Total RNA Extraction Kit [Mini] (Plant)	100 preps/kit, (RB, PRB, Wash, W1) Buffer, RNase-free Water, etc.
		YRPM10	L	Total RNA Extraction Kit [Maxi] (Plant)	10 preps/kit, (RB, PRB, Wash, W1) Buffer, RNase-free Water, etc.
	96-Well	YRB96B-2	-	96-Well Total RNA Extraction Kit	2 pcs/plate, (RB, Wash, W1) Buffer, Adhesive Film, etc.
		YRB96B-4	-	96-Well Total RNA Extraction Kit	4 pcs/plate, (RB, Wash, W1) Buffer, Adhesive Film, etc.
		YRB96B-10	-	96-Well Total RNA Extraction Kit	10 pcs/plate, (RB, Wash, W1) Buffer, Adhesive Film, etc.
	Other	YVM96	-	Vacuum Manifold	Maximum Operating Vacuum: 28 in. Hg
		YPK10	-	Proteinase K	Proteinase K (10 mg/vial)
YRN02/YRN15		-	RNase A	RNase A (50 mg/ml), 0.2 ml / RNase A (50 mg/ml), 1.5 ml	
YLY20		-	Lysozyme	Lysozyme (20 mg/vial)	

Notes

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