miRNA Isolation Kit

For research use only

www.geneaid.com

Sample : 100 mg of tissue or cultured cells (up to 1 x 10^6)

Reactions : 100

Storage : room temperature

Operation time : 30 minutes

Format : spin column

Introduction

The miRNA Isolation Kit was designed for purifying micro RNAs (miRNAs) and other small cellular RNAs from tissue samples or cultured cells. Purification of miRNAs allows for research into biologically significant pathways for gene regulation. Standard protocols for isolating total RNA and mRNA are not optimized for isolation of small RNA molecules and result in the loss of substantial amounts of miRNAs and other small RNA. In addition, removal of the predominantly lager RNAs is required for accurate analysis of miRNA expression by qPCR or microarray analysis. This kit ensures purification of small RNA with minimal contamination from large RNA molecules or genomic DNA. In the presence of chaotropic salt, and various ethanol concentrations in the solvent, RNA molecules (of various sizes) are selectively bound by the glass fiber matrix (1).

Kit Contents

Name	PU009004	PU009100
Lysis Buffer	1 ml	25 ml
Mi Buffer	1.5 ml	3 ml
Wash Buffer[1] (Add Ethanol)	250 µl (1 ml)	12.5 ml (50 ml)
Release Buffer	1 ml	6 ml
RNA Column	8 pcs	200 pcs
2 ml Collection Tube	8 pcs	200 pcs

Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	RBM10/25
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Maxi Kit (Tissue)	10/25 preps	RTM10/25
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Total RNA Maxi Kit (Plant)	10/25 preps	RPM10/25
miRNA Isolation Kit	100 preps	PU009100
96-Well Total RNA Kit	2/4/10 x 96 Wells	RBP02/04/10

[1]Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Additional requirements

absolute ethanol, ddH_20 saturated phenol, chloroform, microcentrifuge tubes

References

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

miRNA Isolation Kit Protocol

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Step	Procedure
Step 1 Lysis	• Add **200 μl of Lysis Buffer** into a 1.5 ml microcentrifuge tube containing 100 mg of tissue or cultured cell pellet (up to 1×10^6). • Vortex vigorously until the pellet is dissolved completely. • Incubate at room temperature for 10 minutes.
Step 2 RNA Precipitation	• Add **20 μl of Mi Buffer** to the 1.5 ml microcentrifuge tube. • Add 180 μl of ddH_2O saturated phenol and 40 μl of chloroform into the 1.5 ml microcentrifuge tube. • Vortex vigorously for 2 minutes. • Centrifuge at 12,000 rpm for 3 minutes. • Transfer the upper phase to a clean 1.5 ml microcentrifuge tube. • Add a 0.35 volume of absolute ethanol to the upper phase and mix well by shaking (eg. add 108 μl of ethanol to 200 μl of upper phase).
Step 3 RNA Binding	• Place a **RNA Column** in a **2 ml Collection Tube** and transfer the ethanol-added mixture to the **RNA Column.** • Incubate for 1 minute. • Centrifuge at 12,000 rpm for 30 seconds. • Transfer the filtrate to a new 1.5 ml microcentrifuge tube (RNase free). • Add a 0.70 volume of absolute ethanol to the filtrate and mix well by shaking (eg. add 676 μl of ethanol to 290 μl of filtrate). • Place a new **RNA Column** in a **2 ml Collection Tube** and transfer the mixture to the **RNA Column**. • Incubate for 1 minute. • Centrifuge at 12,000 rpm for 30 seconds to allow the miRNA to bind to the **RNA Column** membrane.
Step 4 Wash	• Add **200 μl of Wash Buffer** (ethanol added) to the **RNA Column** and incubate for 1 minute. • Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid. • Place the **RNA Column** in a clean 1.5 ml microcentrifuge tube.
Step 5 Elution	• Add **50 μl of Release Buffer** (preheated to 65ºC) into the center of the **RNA Column** and incubate for 3 minutes. • Centrifuge at 12,000 rpm for 3 minutes to recover the miRNA. {Note: The purified miRNA can be further concentrated by a standard ethanol precipitation procedure and then re-dissolved in a small volume of ddH_2O or TE (pH 8.0)}
Step 6 Sample-QC Analysis	• Use a 1/5 volume to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its quality. 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.