miRNA Isolation Kit

For research use only

Sample: up to 100 mg of tissue, up to 1 x 10^6 cultured cells

Format: phenol/chloroform/spin column purification

Operation Time: within 30 minutes

Elution Volume: 50-100 µl

Storage: dry at room temperature (15-25ºC)

Introduction

The miRNA Isolation Kit provides a quick and easy spin column system for purifying and enriching micro RNAs (miRNAs) and other small cellular RNAs from a wide variety of tissue and cells. Since miRNAs are vital for regulating gene expression, this kit is optimized for isolation of small RNA molecules while removing larger RNAs and minimizing genomic DNA contamination for improved sensitive downstream applications.

Quality Control

The quality of the miRNA Isolation Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Purified miRNA is resolved in 50 µl of Release Buffer and a 1/10 volume aliquot (5 µl) is analyzed by electrophoresis on a 2% agarose gel.

Kit Contents

Component	IB47370	IB47371
Lysis Buffer	1 ml	12 ml
Mi Buffer	1.5 ml	1.5 ml
Wash Buffer[1] (Add Ethanol)	250 µl (1 ml)	12.5 ml (50 ml)
Release Buffer	1 ml	6 ml
RNA Columns	8	100
2 ml Collection Tubes	8	100
Micropestle	4	50

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Steps to prevent RNase contamination

Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.

IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

• Additional Requirements: Trypsin, phosphate-buffered saline (PBS), ddH_20 saturated phenol, chloroform, absolute ethanol, microcentrifuge tubes, pipette tips, (optional) RNase-free water

miRNA Isolation Kit Protocol

Sample Preparation	**Tissue** • Remove frozen tissue samples from storage or excise fresh tissue samples. • Transfer up to **100 mg of fresh or frozen tissue** to a 1.5 ml microcentrifuge tube then proceed to Step 1 Lysis. NOTE: Ensure frozen tissue does not thaw prior to adding Lysis Buffer. **Adherent Cultured Animal Cells** • Remove the culture medium and wash cells in PBS. • Aspirate PBS then add 0.10-0.25% Trypsin in PBS. • Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube. • Proceed with Suspension Cultured Animal Cells. **Suspension Cultured Animal Cells** • Transfer cells (up to 1 x 10^6) to a 1.5 ml microcentrifuge tube. • Harvest by centrifugation for 5 minutes at 300 x g. • Carefully remove the supernatant completely by aspiration then proceed to Step 1 Lysis.
Step 1 Lysis	**Tissue:** Add **200 µl of Lysis Buffer** then use a **Micropestle** to grind the tissue until it is dissolved completely. **Cultured cell pellet:** Add **200 µl of Lysis Buffer** then vortex vigorously until the pellet is dissolved completely. • Incubate at room temperature for 10 minutes. At this time, pre-heat the required **Release Buffer** (50 µl/sample) to 65ºC (for Step 5 Elution).
Step 2 RNA Precipitation	• Add **20 µl of Mi Buffer**, **180 µl of ddH_2O saturated phenol and 40 µl of chloroform**. • Vortex vigorously for 2 minutes then centrifuge at 14-16,000 x g for 3 minutes. • Transfer the upper phase to a clean 1.5 ml microcentrifuge tube. • Add a **35% volume of absolute ethanol to the upper phase** and mix well by shaking vigorously. If the upper phase volume is 200 µl, 108 µl of absolute ethanol should be added, e.g. 108/(200+108)=0.35.
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 at room temperature then centrifuge at 14-16,000 x g for 30 seconds. • Transfer the filtrate to a new 1.5 ml microcentrifuge tube. • Add a **70% volume of absolute ethanol to the filtrate** and mix well by shaking vigorously. If the filtrate volume is 290 µl, 676 µl of absolute ethanol should be added, e.g. 676/(290+676)=0.70. • Place a new **RNA Column** in a **2 ml Collection Tube** then transfer the mixture to the **RNA Column**. • Incubate for 1 minute at room temperature. • Centrifuge at 14-16,000 x g for 30 seconds to allow the miRNA to bind to the **RNA Column** membrane.
Step 4 Wash	• Add **200 µl of Wash Buffer** (make sure ethanol was added) to the **RNA Column**. • Incubate for 1 minute at room temperature. • Centrifuge at 14-16,000 x g for 1 minute to completely remove the liquid residue. • Place the **RNA Column** in a clean 1.5 ml microcentrifuge tube.
Step 5 Elution	• Add **50 µl of Release Buffer** (pre-heated to 65ºC) into the CENTER of the **RNA Column**. • Incubate for 3 minutes at room temperature. • Centrifuge at 14-16,000 x g for 3 minutes to recover the miRNA. The purified miRNA can be further concentrated using a standard ethanol precipitation procedure then redissolved in a small volume of RNase-free qater.
QC Analysis	Use a 1/5 volume to run on a polyacrylamide gel to check the 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.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	• Insufficient disruption and/or homogenization • Too much starting material • Centrifugation temperature was too low (should be 20ºC to 25ºC)
Low RNA Yield	• Insufficient disruption and homogenization • Too much starting material • RNA still bound to the RB Column membrane • Ethanol carryover (Solution: after adding 200 µl of Wash Buffer to the RNA Column and centrifuging at 14-16,000 x g for 1 minute, discard the flow-through then place the RNA Column back in the 2 ml Collection Tube. Centrifuge the RNA column at 14-16,000 x g for another 3 minutes to dry the column membrane and remove the residual ethanol.
RNA Degradation	• Harvested sample not immediately stabilized • Inappropriate handling of starting material • RNase contamination