User's Manual and Instructions

Product: Broad Range Total RNA Isolation Kit (for Co-Purifying Small and Large RNAs)

Catalog Number: K1341050

Introduction

Spin column based RNA isolation method is routinely used, however, does not effectively recover small RNAs (< 200 nt). BioChain's Broad Range Total RNA Isolation Kit provides a rapid method for co-purifying small RNAs (< 200 nt) and large RNAs (> 200 nt) from tissue culture cells and small tissue samples. These small RNAs include regulatory RNA molecules such as microRNAs (miRNAs) and short interfering RNAs (siRNA), as well as tRNAs and 5S rRNAs. The RNAs isolated using BioChain's Broad Range Total Isolation Kit can be used in various downstream applications relating to gene regulation and functional analysis, including qRT-PCR, northern blotting and microarray profiling analysis.

Features

- **Simple** – quick and easy protocol using rapid spin-column format, can isolate total RNA in less than 30 min.
- **Versatile** – isolate both large RNAs and small RNAs
- **High quality** – isolated RNAs can be used for various downstream applications
- **Reliable**: repeatable, minimal contamination from genomic DNA

Applications

- Isolation of total RNA including small RNAs (< 200nt) and large RNAs (> 200 nt) from tissue culture cells and small tissue samples.

Description

BioChain's Broad Range Total RNA Isolation Kit provides a rapid method for isolate total RNAs [including small RNAs (< 200 nt) and large RNAs (> 200 nt)] from tissue culture cells and small tissue samples. This kit contains enough reagents for 50 isolations of total RNAs.

Column binding capacity	100 μg
Recommended amount of starting material for one isolation: Solid tissues Tissue culture cells	 0.5 – 200 mg 100 – 1x 10^7
Maximum column loading volume	650 μl
Elution Volume	30 – 100 μl

Quality Control

A representative kit from the same lot is randomly selected for isolation of total RNAs from cultured cells and tissues The quality and purity of isolated total RNAs were measured by denaturing agarose gel electrophoresis and spectrophotometer. $A_{260/280}$ is between 1.8 and 2.0 (measured in 10 mM Tris-Cl, pH 7.5). The presence of miRNAs in the isolated total RNA was detected using BioChain's MicroRNA One-Step qRT-PCR Detection Kit (Cat# KS081200 and Cat# KS082200) (Figure 1).

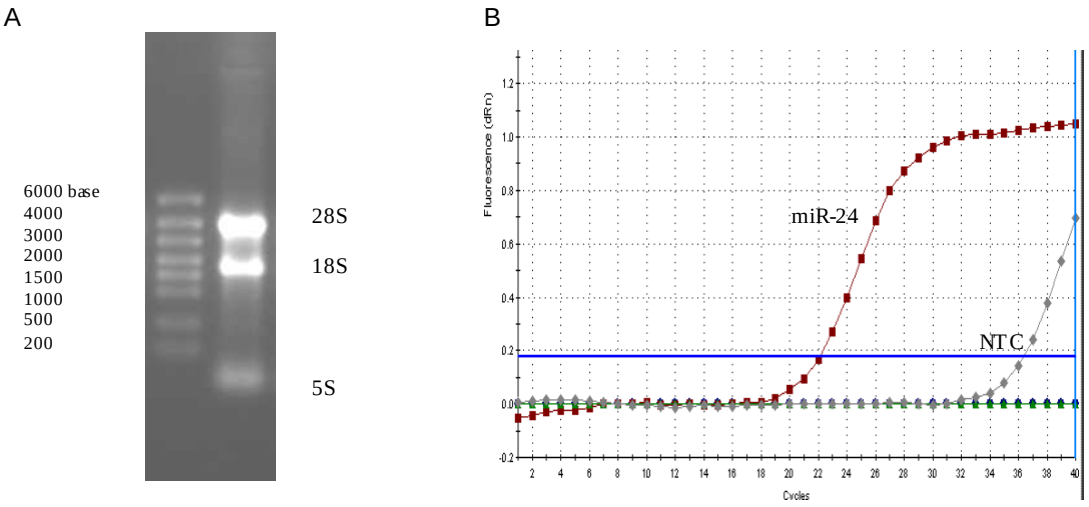

Fig. 1 A. Isolated total RNA from human placenta tissue was analyzed by electrophoresis on 1% denaturing agarose gel. Lane 1: RNA ladder. Lane 2: isolated human placenta total RNA.
B. Detection of the presence of miRNAs in 25 ng total RNAs isolated from human placenta using BioChain's miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200)

Kit Components

Item	Part No.	Amount	Storage
1. Lysis Buffer	K1341050-1	50 ml	4°C
2. Lysis Enhancer Solution	K1341050-2	5 ml	Room Temp
3. Acidic Phenol:Chloroform	K1341050-3	60 ml	4°C
4. Spin Column with Collection Tubes	K1341050-4	50	Room Temp
5. Wash Buffer 1	K1341050-5	35 ml	Room Temp
6. Wash Buffer 2 (Concentrated)	K1341050-6	20 ml	Room Temp
7. RNase-Free Water	K1341050-7	6 ml	Any Temp
8. Elution Buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA)	KS341025-8	6 ml	Any Temp

This kit provides enough reagents for isolation of total RNAs from 10 g tissue.

Items not supplied

1. 100% Ethanol
2. RNase-Free Microcentrifuge Tubes

Storage and Stability

Store the solutions at the appropriate temperature. The kit is stable for one year when handled properly.

Protocol

I. Things to Do Prior to Use

Add 80 ml of 100% ethanol to the bottle containing the concentrated Wash Buffer 2. Mix well. This will give a final volume of 100 ml. Mark the bottle to indicate that the ethanol has been added.

II. Preparing Tissue Culture Cell Lysate

This protocol is optimized for isolating RNA from 100 – 1x 10^7 cells.

Cell number	Amount of Lysis Buffer Needed
100 – 1x 10^5	350 µl
1x 10^5 – 1x 10^7	650 µl

1. Harvest cells using the method appropriate to the properties of the cell line. For adherent cells, trypsinize the cells using standard techniques. Count the cell number.
2. Pelleting the cells by centrifuging at 200 – 300 g for 5 min. Carefully remove the supernatant by aspiration.
3. Wash the pellet once with ice-cold PBS. Pelleting the cells by centrifuging at 200 – 300 g for 5 min. Carefully remove the supernatant by aspiration. Keep the pellet on ice.
4. Add appropriate volume of Lysis Buffer to the cell pellet. Vortexing until the pellet is completely lysed.
5. Transfer the lysate into a RNase-free microcentrifuge tube. Keep the tube on ice and proceed to the protocol in Section IV: Organic Extraction to Remove DNAs and Proteins.

III. Preparing Tissue Lysate

This protocol is optimized for isolating RNA from 0.5 – 200 mg tissue.

1. Weigh the tissue and mince the tissue into smaller pieces. Place the tissue in a Rnase-free tube.
2. Add 10 volumes per tissue mass of Lysis Buffer to the tissue (for example, adding 1 ml Lysis Buffer for 100 mg tissue). Keep the tube on ice.
3. Homogenize the tissue using a homogenizer (such as Polytron homogenizer, Knotes pellet pestle, or Dounce homogenizer). Keep the lysate on ice and proceed to the protocol in Section IV: Organic Extraction to remove DNAs and Proteins.

IV. Organic Extraction to Remove DNAs and Proteins

1. Add a volume of Lysis Enhancer Solution equal to 1/10 volume of the cell or tissue lysate (For example, adding 65 µl Lysis Enhancer Solution to 650 µl lysate). Mix well by shaking vigorously for 15 sec.
2. Add a volume of Acidified Phenol:Chloroform equal to 1.2 volume of the cell or tissue lysate (For example, adding 780 µl Acidified Phenol:Chloroform to 650 µl cell or tissue lysate). Mix well by shaking vigorously for 30 sec.
3. Centrifuge at 16,000 g (typically ~13,000 rpm at a microcentrifuge) at 4°C for 10 min to separate the aqueous phase (upper phase) and the organic phase (lower phase). Some protein material may be visible at the interphase layer.
4. Carefully remove the upper aqueous phase containing RNAs to a new RNase-free microcentrifuge tube. Note the volume of the aqueous phase recovered.
5. Proceed to the protocol in Section V: Purification of Total RNAs

V. Purification of Total RNAs

1. To the tube containing the aqueous phase, add a volume of 100% ethanol equal to 1.25 volume of the aqueous phase (the final ethanol concentration is 55.6%) (For example,

adding 270 µl 100% ethanol to 216 µl aqueous phase to make the final total volume at 486 µl). Mix well by vigorously shaking for 15 sec.

2. Place a spin column in the supplied collection tube. Load 650 µl mixture from step 1 to the spin column. Cap the spin column.
3. Centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
4. Remove and retain the spin column and discard the flowthrough.
5. If the sample volume was greater than 650 µl in step 2, re-seat the spin column in the collection tube and repeat steps 2– 4 with the remaining mixture.
6. Add 650 µl Wash Buffer 1 to the spin column. Close the lid and centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
7. Remove and retain the spin column and discard the flowthrough.
8. Replace the spin column in the collection tube. Add 650 µl Wash Buffer 2 to the spin column. Close the lid and centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
9. Discard the flowthrough and repeat step 8 once.
10. Discard the flowthrough and the collection tube. Place the spin column in a new microcentrifuge tube. Open the lid and centrifuge at 16.000 g (~13,000 rpm) at room temperature for 1 min.
11. Place the spin column in a new RNase-free microcentrifuge tube, add 30 – 100 µl Elution Buffer or Rnase-Free Water to the spin column membrane. Close the lid and centrifuge at 16,000 g (~13,000 rpm) at room temperature for 1 min to elute the RNAs.
12. The purified RNA sample may be placed at –20°C for short time storage or –70°C for long term storage.

VIII. Analysis of RNA after Purification

1. Measuring the absorbance at A_{260} to quantify RNA based on the RNA extinction coefficient (1 unit A_{260} = 40 ng/ul). The ratio of A_{260} to A_{280} provides an indication of RNA purity. For RNA of good quality and purity, the ratio of A_{260} /A_{280} is between 1.8 – 2.
2. An alternative way to access RNA quantity and quality is to run a RNA sample on a denaturing acrylamide or agarose gel.
3. For detection of the presence of miRNAs in isolated total RNA samples, it is recommended to use BioChain's miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200) or miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200). We also provide custom service to design qRT-PCR primer set for specific miRNAs for using BioChain's MicroRNA One-Step qRT-PCR Detection Kit.

Related Products

MicroRNA Isolation Kit (Cat# KS341025); miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200); miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200).

References

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2. Lim, L. P. *et al. Science* 2003. 299(5612):1540.
3. Boom R. *et al. Journal of Clinical Microbiology* 1990. 23(3): 495-503.
4. Chomczynski P. and Sacchi N. *Analytical Biochemistry.* 1987. 162:156-159.