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User's Manual and Instructions

MicroRNA Isolation Kit

Catalog Number: KS341025

Introduction

MicroRNAs (miRNAs) are small, highly conserved non-coding RNA molecules (approximately 19 – 23 nt). miRNAs are involved in the regulation of translation and degradation of target mRNAs, through base pairing to complementary or partially complementary sites in the 3'-untranslated regions of mRNAs. miRNAs play very important role in the regulation of development, cell proliferation, apoptosis, and differentiation. BioChain's MicroRNA Isolation Kit provides a rapid method for the isolation and purification of small RNA molecules (<200 nt) from tissue culture cells, small tissue samples, and bio-fluid. These small RNAs include regulatory RNA molecules such as miRNAs and short interfering RNAs (siRNAs), as well as tRNAs and 5S rRNAs. The small RNA molecules isolated using BioChain's MicroRNA 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. This kit can also be used to isolate both large RNAs (>200 nt) and small RNAs (<200 nt).

Features

- **Simple** – quick and easy protocol using rapid spin-column format, can isolate miRNA in less than 30 min.
- **Versatile** – isolate all small RNAs (<200 nt), and can also be used for isolate large RNAs (>200 nt).
- **High quality** – isolated small RNAs can be used for various downstream applications.
- **Reliable**: repeatable, minimal contamination from large RNA and genomic DNA.

Applications

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

Description

BioChain's MicroRNA Isolation Kit provides a rapid method for the isolation and purification of small RNA molecules (<200 nt) not only from tissue culture cells, and tissues, but also from blood and serum. This kit can also be used for isolating large RNA (>200 nt). This kit contains enough reagents for 25 isolations of small RNAs (<200 nt) or 50 isolations of large RNAs (>200 nt).

Column binding capacity	100 μg
Recommended amount of starting material for one isolation: Tissues Tissue culture cells Blood and serum	 0.5 – 200 mg 100 – 1x 10^7 200 μl
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 small RNAs from cultured cells and tissues. The quality and purity of isolated small 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 small RNAs was detected using BioChain's MicroRNA One-Step qRT-PCR Detection Kit (Cat# KS081200 and Cat# KS082200) (Figure 1).

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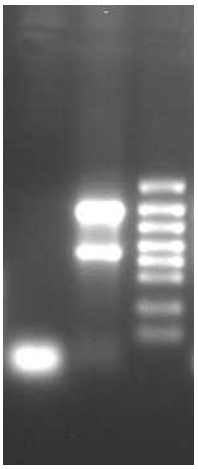
A



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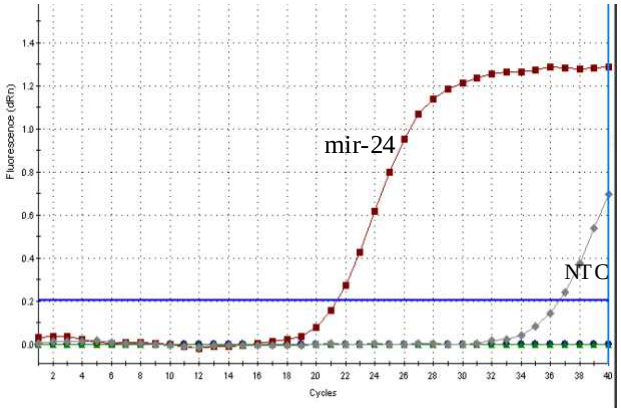

Fig. 1 A. Isolated small RNAs and large RNAs were analyzed by electrophoresis on 1% denaturing agarose gel. Large RNAs and small RNAs were isolated from one human placenta tissue lysate. Lane 1: purified small RNAs. Lane 2: purified large RNAs. Lane 3. RNA ladder.
B. Detection of the presence of miRNAs in 1 ng small 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	KS341025-1	50 ml	4°C
2. Lysis Enhancer Solution	KS341025-2	5 ml	Room Temp
3. Acidified Phenol:Chloroform	KS341025-3	60 ml	4°C
4. Spin Column with Collection Tubes	KS341025-4	50	Room Temp
5. Wash Buffer 1	KS341025-5	35 ml	Room Temp
6. Wash Buffer 2 (Concentrated)	KS341025-6	20 ml	Room Temp
7. RNase-Free Water	KS341025-7	6 ml	Any Temp
8. Elution Buffer (10mM Tris-HCl, pH 7.5, 0.1mM EDTA)	KS341025-8	6 ml	Any Temp

This kit provides enough reagents for isolation of small RNAs from 5 g tissue or isolation of large 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 V: 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 V: Organic Extraction to remove DNAs and Proteins.

IV. Preparing and treatment of the liquid sample

1. Serum or Plasma (fresh or from -80°C) is spin at 10,000 rpm for 5 min to remove the partial or cells may contaminate in the sample. Transfer 200 µl of sample into a new 1.5 ml tube.
2. Add 200 µl of Lysis Buffer to the sample. Vortexing until mix well.
3. Add 40 µl of Lysis Enhancer Solution and mix well by shaking vigorously for 15 sec.
4. Add 440 µl of Acidified Phenol:Chloroform equal to and mix well by shaking vigorously for 30 sec.
5. 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.
6. Carefully remove the upper aqueous phase, ~400 µl, containing RNAs to a new RNase-free centrifuge tube. Keep the tube on ice and proceed to the protocol in Section VI: Purification of the Total RNA including the Small RNAs

V. 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 centrifuge tube. Note the volume of the aqueous phase recovered.
5. Proceed to the protocol in Section VII: Separation of Small RNAs and Large RNAs.

VI. Purification of the Total RNA including the Small RNAs

1. Add 1 ml of 100% ethanol (the final ethanol concentration is about 70%). Mix well by vigorously shaking for 15 sec.
2. Place a spin column in a collection tube. Load 700 µl mixture to the new spin column. Close the lid and centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
3. Remove and retain the spin column and discard the flowthrough.
4. Load the left sample, about 700 µl, re-seats the spin column in the collection tube and repeat steps 3– 4 with the remaining mixture.
5. Place the spin column in the collection tube, add 650 µl Wash Buffer 2 to the spin column and close the lid. Centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
6. Discard the flowthrough and repeat step 6 twice.
7. 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.
8. Place the spin column in a new RNase-free microcentrifuge tube; add preheated (90^{o}C) 50 – 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 small RNAs.
9. The purified RNA sample may be placed at –20°C for short time storage or –70°C for long term storage.

VII. Separation of Small RNAs and Large RNAs

1. To the tube containing the aqueous phase, add a volume of 100% ethanol equal to 0.43 volume of the aqueous phase (the final ethanol concentration is 30%) (For example, adding 215 µl 100% ethanol to 500 µl aqueous phase). Mix well by vigorously shaking for 15 sec.
2. Place a spin column into 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. Retain the flowthrough, which contains the small RNAs.
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. If purifying small RNAs only, discard the spin column and follow the protocol in purification of small RNAs. If also purifying large RNAs, replace the spin column into the collection tube and save it for use in Section IX: Purification of Large RNAs (the spin column can be stored at 4°C, but not for long periods).

VIII. Purification of Small RNAs

1. Pipet the flowthrough from step 4 in section VII to a new microcentrifuge tube. Note the volume of the flowthrough.
2. Add a volume of 100% ethanol equal to 1.2 volume of the flowthrough (the final ethanol concentration is 70%). Mix well by vigorously shaking for 15 sec.
3. Place a new spin column in a new collection tube. Load 650 µl mixture to the new spin column. Close the lid and 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 3, re-seat the spin column in the collection tube and repeat steps 3– 5 with the remaining mixture.
6. Place the spin column in the collection tube, add 650 µl Wash Buffer 2 to the spin column and close the lid. Centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
7. Discard the flowthrough and repeat step 6 twice.
8. 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.
9. 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 small RNAs.
10. The purified RNA sample may be placed at –20°C for short time storage or –70°C for long term storage.

IX. Purification of Large RNAs

1. Add 650 µl Wash Buffer 1 to the first spin column (which binds Large RNAs) from step 6 in Section VII. Close the lid and centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
2. Remove and retain the spin column and discard the flowthrough.
3. 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.
4. Discard the flowthrough and repeat step 3 once.
5. 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.
6. 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 large RNAs.
7. The purified RNA sample may be placed at –20°C for short time storage or –70°C for long term storage.

X. Isolation of Small RNAs from Total RNA

This kit can be partially used for isolating small RNAs from total RNAs which is produced from other methods, the conditions are as follows:

1. If the total RNAs is already dissolved in DEPC water or any solution already, then add 5 volume of lysis buffer as aqueous phase in step V, and start from step V
2. If the total RNAs is still in pellet stage, dissolve the total RNAs with lysis buffer of this the kit as aqueous phase in step V, and start from step V.
3. If the RNAs is very diluted and has very large volume, therefore make the 5 volume lysis buffer too much, then precipitate the total RNAs and redissolve it into lysis buffer of this kit as aqueous phase in step V, and start from step V.

XI. 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/μl). 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 – 2.0
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 small 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

miR-24 One-Step qRT-PCR Detection Kit (Cat# KS081200). miR-16 One-Step qRT-PCR Detection Kit (Cat# KS082200). Broad Range Total RNA Isolation Kit for Co-Purifying Large and Small RNAs (Cat# K1341050).

References

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