Signosis
Innovative Plate Assay Solutions

miRNA Real-Time PCR Assay Kit

Catalog Number CL-0004 **(For Research Use Only)**

Introduction

miRNAs are known to involve in multiple biological functions and abnormal expression is associated to human diseases such as cancers. They represent a promising new class of cancer biomarkers. A number of PCR methods have been developed for analyzing the expression level of miRNA. Based on its proprietary technology, Signosis has developed a highly sensitive and discriminative real-time PCR assay method for measuring miRNA expression. It implements oligo-ligation and SYBR green based real-time PCR. The assay can be used for quantitative analysis of miRNA expression in either total RNA or cell lysate without cDNA conversion.

Principle

In the assay, the target miRNA molecule is hybridized with two oligos to form a RNA/DNA duplex. When the sequences are perfectly matched, they are aligned with the miRNA and the joint can be ligated with DNA ligase. A single nucleotide difference among miRNAs will block either the hybridization or the ligation. After the pair of oligos is ligated, the ligated molecules are subjected to real-time PCR analysis.

Materials provided

- Annealing buffer (RT)
- Magnetic streptavidin beads (4 °C)
- Beads binding buffer (RT)
- Bead wash buffer (RT)
- T4 DNA Ligase (-20°C)
- Ligation buffer (-20°C)
- SYBR Green PCR Master Mix (-20°C)

Material required but not provided

- Cell lysis buffer (CL-0001, Signosis)
- miRNA oligo mix-PCR and Specific miRNA qPCR primer (PO-0XXX, Signosis)
- Magnetic stand (96 well plate)
- RNase free water
- Real time PCR machine
- 0.2ml PCR tube or plate

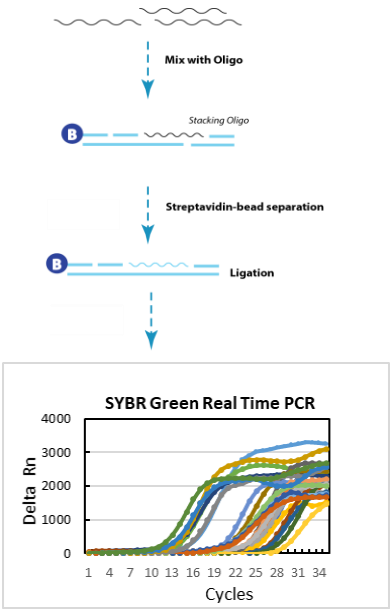

Diagram of miRNA Real-time PCR Assay

1. Sample preparation procedure

Total RNA or cell lysate can be used for the assay

(1) Total RNA preparation

We recommend using Trizol reagent or similar products. The total RNAs are precipitated with isopropanol or ethenol along with small RNAs including miRNAs.

(2) Cell lysate

a. Estimate the number of cells. The number of cells should be between 10^4-10^5 cells. Wash the cells with 200ul ice cold 1XPBS and add 100ul ice-cold Cell lysis buffer and then subject to snap-frozen at -80°C. If the cell number is between 2000-10,000 cells, add 50ul Cell lysis buffer instead.

Notes: Keep the cells on ice during the procedure.

b. Incubate for 10 minutes on ice, and centrifuge at 10,000g for 2 minutes. Transfer the supernatant to a fresh tube.

Optional: Add 0.25-1u DNAse I, and incubate at 37 °C for 10 minutes and inactivate at 75 °C for 10 minutes.

c. Heat the supernatant for 75 °C for 15 minutes and put on ice. The cell lysate is ready for use or can be stored at -80°C for the future usage.

2. miRNA/oligo Selection and Formation

(1) Reaction preparation to form miRNA/oligo hybrid

X µl 100ng-2µg total RNA or cell lysate
2 µl specific miRNA oligo mix
15 µl Annealing buffer
X µl ddH2O

30ul

(2) Incubate on a PCR machine at 72ºC for 5 minutes and 53ºC for 60 minutes.

(3) Beads washing

- Resuspend the beads by gently tapping the tube to obtain a homogeneous suspension.
- Transfer 4 µl beads to a 0.2ml PCR tube (the size of the tube that should fit into the magnetic stand.
- Add 50 µl of annealing buffer to the tube, place onto the magnetic stand for 30 seconds.
- Aspirate out the liquid.
- Remove the tube from magnetic stand.

(4) Beads selection

- Add 30 µl of Bead binding buffer to 30 µl annealed miRNA/oligo hybrid From Step2(1), transfer to the washed beads and resuspend the beads in the solution.
- Incubate at 37ºC for 30 minutes.
- Place the bead mixture on the magnetic stand for 30 seconds and aspirate out the buffer. The beads will remain on the side of tube.
- Remove the tube from the magnetic stand, add 100 µl of Bead wash buffer, pipette gently up and down to resuspend the beads, place the tube on the magnetic stand for 30 seconds and then aspirate the buffer. Repeat the washing step once.

3. Ligation of miRNA-directed oligos to form a single molecule

(1) Add 50 µl of Ligation buffer to resuspend the beads, pipette gently up and down, place the tube on the magnetic stand for 30 seconds, and aspirate the buffer.

(2) Remove the tube from the magnetic stand. Add 20 µl of ligation buffer to resuspend the beads, then add 1µl ligase to the resuspended beads and incubate at 37º for 90 minutes.

(3) Add 100 µl Bead washing buffer directly to 20 µl ligation reaction mix from Step 3(2), place the tube on the magnetic stand for 30 seconds, and aspirate the buffer.

(4) Remove the tube from the magnetic stand, add 20ul ddH_2O and resuspend the beads in 20ul ddH_2O and heat at 95ºC for 3 minutes on a PCR machine with heated lid to release the ligated molecule from the beads.

(5) Place the reaction tube on the magnetic stand for 30 seconds. Immediately transfer the solution to a fresh tube.

4. Real-time PCR

(1) Mix the following component for one reaction:
20 µl SYBR Green PCR Master Mix
1 µl miRNA qPCR primer
1 µl ligated molecule

(2) Proceed PCR cycles:
Heating the reaction at 95 ºC for 30 seconds.
Proceed two-step PCR 35 cycles
95 ºC 15 seconds
55 ºC 30 seconds
72 ºC 30 seconds

(3) Proceed data analysis

Data of example

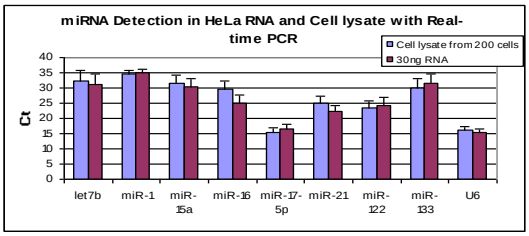

Figure 1. miRNA detection in HeLa RNA and Cell lysate. HeLa total RNA was prepared with Trizol reagent. HeLa cell lysate from 200 cells was prepared with Cell Lysis Buffer according to manual. The samples were subjected to miRNA real time PCR assay on a 7700 system.