Signosis
Innovative Plate Assay Solutions

miR-17-92 Cluster Real-Time PCR Assay Kit

Catalog Number CL-0005 **(For Research Use Only)**

Introduction

The miR-17-92 cluster encoding miR-17-3p, miR-17-5p, miR-18a, miR-19a, miR-19b, miR-20a and miR-92 is highly expressed in tumor cells and are known to act as oncogenes. Expression of these miRNAs promotes cell proliferation, suppresses apoptosis of cancer cells, and induces tumor angiogenesis. New work reveals essential functions for these miRNAs not only in tumor formation but also during normal development of the heart, lungs, and immune system. 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 multiple miRNA expression simultaneously. It implements oligo-ligation and SYBR green based real-time PCR. The assay can be used for quantitative analysis of miRNA expression of miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-19b, miR-20a, miR-92 and U6 in either total RNA or cell lysate without cDNA conversion.

Principle

In the assay, a target miRNA molecule is hybridized with two oligos to form a RNA/DNA duplex. When the sequences are perfectly matched, these two oligos are aligned with the miRNA target. The joint of the two oligos 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. In addition, a unique tag sequence is assigned to one specific miRNA. Different isoforms such as miR-19a and miR-19b can be differentiated during PCR when the tag sequence is used as one of amplification primers.

Material required but not provided

- Cell lysis buffer (CL-0001, Signosis)
- Magnetic stand
- RNase free water
- Real time PCR machine
- 0.2ml PCR tube or plate

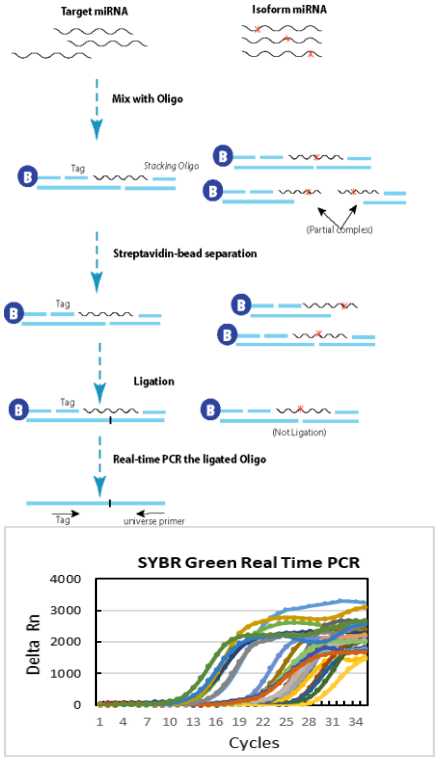

Diagram of miR-17-92 Real-time PCR Assay

Materials provided

- miR-17-92 Oligo Mix
- Annealing buffer (RT)
- Magnetic streptavidin beads (4 ºC)
- Beads binding buffer (RT)
- Bead wash buffer (RT)
- T4 DNA Ligase (-20ºC)
- Ligation buffer (-20ºC)
- 2X SYBR Green PCR Master Mix (-20ºC)
- 7 miRNA specific primers and U6 Primer (-20ºC)

1. Sample preparation procedure

Either total RNA or cell lysate can be used for the assay.

(1) Total RNA preparation

- We recommend using Trizol reagent or similar products to prepare total RNA. Small RNAs including miRNAs are co-precipitated with other sizes of RNA with isopropanol or ethanol of total RNA.

(2) Cell lysate

- Estimate the number of cells. The number of cells should be between 10^4-10^5 cells. Wash the cells with 200 µl ice cold 1X PBS and add 100 µl ice-cold cell lysis buffer and the snap freeze at -80ºC. If the cell number is between 2000-10,000 cells, add 50 µl cell lysis buffer instead.
    - **Notes**: Keep the cells on ice during the procedure.
- Incubate with cell lysis buffer on ice for 10 minutes, and centrifuge at 10,000g for 2 minutes. Transfer the supernatant to a fresh tube
- Optional: Add 0.25-1µl DNAse I, and incubate at 37 ºC for 10 minutes and inactivate at 75 ºC for 10 minutes.
- Heat the supernatant for 75 ºC for 15 minutes, and put on ice. The cell lysate is ready for use or can be stored at -80ºC for the future use.

2. Annealing of oligos with miRNA

(1) Annealing reaction:

50 ng- 5µg Total RNA or Cell Lysate	50 - 100 µl from Total Cell Lysate
miRNA oligo mix	3 µl
Annealing Buffer	15 µl
ddH2O	X µl
	30 µl Total

(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 Step 2-(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 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 annealed oligos

(1) Equilibrate beads with 50 µl of Ligation buffer and 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. 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. The ligation mixture is ready to use.

4. Real-time PCR

(1) Mix the following components for one reaction

2X SYBR Green PCR Master Mix	10 µl
Specific miRNA Primer	1 µl
Ligated Molecule	2 - 1 µl
ddH2O	X µl
	X µl

Note: The master mix can be made by multiplying the volume with reaction number, and then add specific miRNA primer

(2) PCR cycles:

Heat the reaction at 95 ºC for 30 seconds.

Proceed three-step PCR 35 cycles

95 ºC	15 seconds
55 ºC	30 seconds
72 ºC	30 seconds

(3) Proceed data analysis

Example Data