Signosis
Innovative Plate Assay Solutions

Apoptosis-Associated MiRNA Plate Array

Catalog Number MA-1002 **(For Research Use Only)**

Introduction

Apoptosis involves a series of biochemical events leading to a characteristic cell morphology and death, including changes to the cell membrane, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. It plays important roles in removing unwanted cells during development and homeostasis. Excessive apoptosis causes hypotrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer. MicroRNAs are newly discovered small non-coding RNA molecules that regulate up to 30% of mammalian gene expression. Numerous miRNAs have been revealed to have an influence on the regulation of apoptotic cell signaling such as overexpression of miR-145, miR-216, miR-182, and miR-96 miRNA to decrease caspase-3 activation. Signosis developed a miRNA array for targeting 47 miRNAs that have been shown in literature to be involved in apoptosis. Profiling the expression of these miRNAs will facilitate in uncovering the regulation of miRNA in apoptosis.

Principle of the assay

Signosis' apoptosis-associated miRNA plate array is a simple two-step assay; plate hybridization and streptavidin-HRP detection. The plate is pre-coated with an oligo mix, including a pair of unique oligos that hybridize side-by-side to a specific target miRNA and a universal capture oligo and a biotin-labeled oligo. In the assay, total RNA is directly utilized for hybridization. When the target miRNA exists in RNA, it acts as a bridge to bring the biotin-labeled oligo to the capture oligo, which can be detected through streptavidin-HRP conjugate and a chemiluminescent substrate. If there exists no specific miRNA, the biotin-labeled probe will be washed away, leading to no detection. In the plate array, 48 wells are coated with different oligo mixes for different miRNAs. A single 96-well plate allows quantitative measurement and comparison of 48 miRNAs between two samples. U6 RNA is used for normalization.

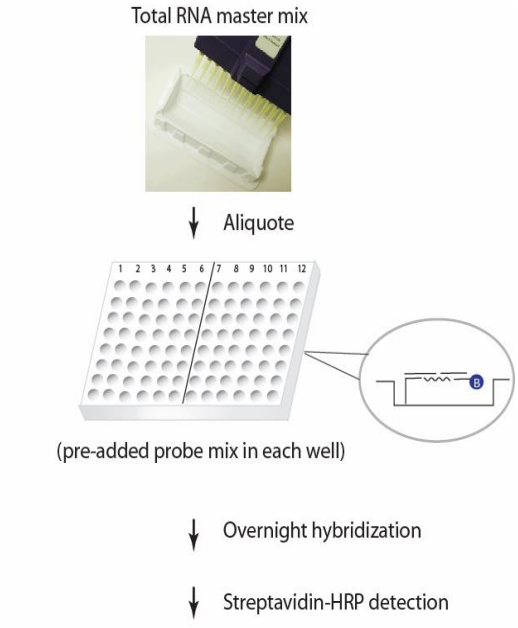

Diagram of miRNA Direct Hybridization Plate Array

Materials provided with the kit

- One 96-well plate (RT)
- Biotin detection oligo (-20°C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)
- Sealing foil

Instrument and materials required

- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection
- 50 ml reagent reservoir

Reagent preparation before starting experiment

- Warm up Plate hybridization buffer and Hybridization Wash buffer at 42°C before use. Stir the solution with 10ml or 5ml pipette to facilitate the dissolving process.
- Dilute 30ml of 5x Plate Hybridization wash buffer with 120 ml of dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute 500 times of streptavidin-HRP with block buffer before use at Step 9.

Assay procedure

1. Remove the sealing film from the plate.

2. Mix the following items in a 50 ml reagent reservoir.
   Briefly spin the tubes before use

   5.5ml Hybridization Buffer
   10-30 µl RNA (10-30 µg)
   2.5ul Biotin Detection Oligo

3. Aliquot 100ul to each well with a multi-channel pipette.

4. Seal the wells with foil film securely and hybridize at 45 °C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*

5. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 200µl of warmed 1x Plate hybridization wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels

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7. Add 200µl of Blocking buffer incubate for 15 minutes at room temperature with gentle shaking.

8. Invert the plate over an appropriate container to remove block buffer.

9. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.

10. Wash the plate 3 times with 1X Detection wash buffer for 5 min at room temperature with gently shaking. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. Repeat for additional 2 time washes.

11. Freshly prepare the substrate solution:
    For the whole plate:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer

12. Add 95µl substrate solution to each well and incubate for 1 min.

13. Place the plate in the luminometer, and read. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Example of Data Analysis

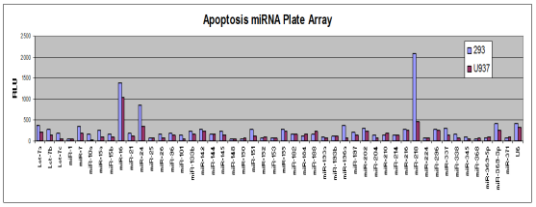

Figure1: Apoptosis-associated miRNA plate array analysis of 48 miRNA expression in HEK293 and U937.

Schematic diagram of Apoptosis-Associated miRNA Plate Array

Let-7a	miR-16	miR-142	miR-155	miR-202	miR-337
Let-7b	miR-21	miR-144	miR-182	miR-204	miR-338
Let-7c	miR-24	miR-145	miR-184	miR-210	miR-345
miR-1	miR-25	miR-148	miR-188	miR-214	miR-368
miR-7	miR-26	miR-150	miR-193a	miR-216	miR-369-5p
miR-10a	miR-96	miR-151	miR-193b	miR-218	miR-369-3p
miR-15a	miR-101	miR-152	miR-196a	miR-224	miR-371
miR-15b	miR-133b	miR-153	miR-197	miR-296	U6