Signosis, Inc.
Innovative Plate Assay Solutions

Stem Cell-Associated MiRNA Plate Array

Catalog Number MA-1003 **(For Research Use Only)**

Introduction

Stem cells can differentiate into all of the specialized embryonic tissues during embryo development. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, in order to maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues. Recent studies suggest that maintenance and differentiation of stem cells are regulated by microRNAs (miRNA). MiRNAs are small single-stranded RNA molecules (~20nt long), playing an important role in gene regulation and cell differentiation. MiRNAs are found to be differentially expressed in stem cells, such as brain-specific miR-124a and miR-9 molecules during neural lineage differentiation. In addition, miRNAs are also reported to regulate cancer stem cells. Signosis has developed a miRNA plate array specifically to target 47 miRNAs that are shown in literature to be associated with stem cell differentiation and maintenance. The array can be used for human, mouse and rat samples. Profiling the expression of these miRNAs will help to reveal the functions of miRNAs in stem cells.

Principle of the Assay

Signosis, Inc.'s *Stem Cell-Associated miRNA Direct Hybridization 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.

Instrument and Materials Required

- Hybridization incubator
- Plate Shaker
- Multichannel Pipette
- Plate reader for chemiluminescent detection
- 50mL reagent reservoir

Materials Provided with the Kit

Component	Qty	Store at
Pre-coated 96-Well Plate	1	4°C
Plate Hybridization Buffer	15mL	RT
5X Plate Hybridization Wash Buffer	30mL	RT
5X Detection Wash Buffer	40mL	RT
Blocking Buffer	30mL	RT
Streptavidin-HRP Conjugate	20µL	4°C
Substrate A	1mL	4°C
Substrate B	1mL	4°C
Substrate Dilution Buffer	8mL	4°C
Biotin Detection Oligo	6µL	-20°C

Reagent Preparation Before Starting Experiment

- Warm up *Plate Hybridization Buffer* and *Hybridization Wash Buffer* at **42°C** before use.
- Dilute **30mL** of *5X Plate Hybridization Wash Buffer* with **120mL** of dH_2O before use.
- Dilute **40mL** of *5X Detection Wash Buffer* with **160mL** of dH_2O before use.
- Make a **1:500** dilution of S*treptavidin-HRP Conjugate* in *Blocking Buffer* before use at Step 8.

Please Read the Assay Procedure Before You Begin

Assay Procedure

1. Remove the sealing film from the plate.

2. Mix the following items in a 50mL reagent reservoir.
   5.5mL *Hybridization Buffer*
   10-30µL *RNA* (10-30µg)
   2.5µL *Biotin Detection Oligo*

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

4. Seal the wells with foil film securely and hybridize at **45°C** 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. 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.

6. Add **200µL** of *Blocking Buffer* incubate for **15 minutes** at room temperature with gentle shaking.

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

8. Add **100µL** of diluted *Streptavidin-HRP Conjugate* to each well and incubate for **45 minutes** at room temperature with gentle shaking.

9. Wash the plate 3 times with *1X Detection Wash Buffer* for **5 minutes** 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 washes.

10. Freshly prepare the substrate solution by mixing **1 part** *Substrate A*, **1 part** *Substrate B*, and **8 parts** *Substrate Dilution Buffer*.
    For example, for the whole plate:
    1mL *Substrate A*
    1mL*Substrate B*
    8mL *Substrate Dilution Buffer*
    10mL *Substrate Solution*

11. Add **95µL** S*ubstrate Solution* to each well and incubate for **1 minute.**

12. 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 minute**s.

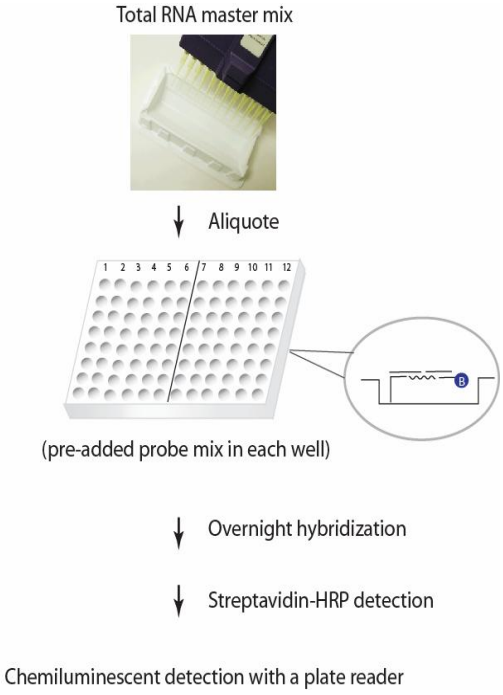

Diagram of miRNA Direct Hybridization Plate Array

Schematic diagram of miRNA Direct Hybridization Plate Array I

Let-7a	miR-16	miR-25	miR-99a	miR-125b	miR-146a
Let-7b	miR-17-5p	miR-26a	miR-101-1	miR-126	miR-149
Let-7c	miR-19a	miR-27a	miR-103	miR-128a	miR-150
miR-1	miR-19b	miR-30a-3p	miR-106a	miR-132	miR-155
miR-9	miR-21	miR-34a	miR-107	miR-133a	miR-181a
miR-10a	miR-22	miR-92b	miR-122a	miR-134	miR-193a
miR-15a	miR-23a	miR-93	miR-124a	miR-135b	miR-218
miR-15b	miR-24	miR-96	miR-125a	miR-141	U6