



Stem Cell-Associated MiRNA Plate Array

Catalog Number MA-1003

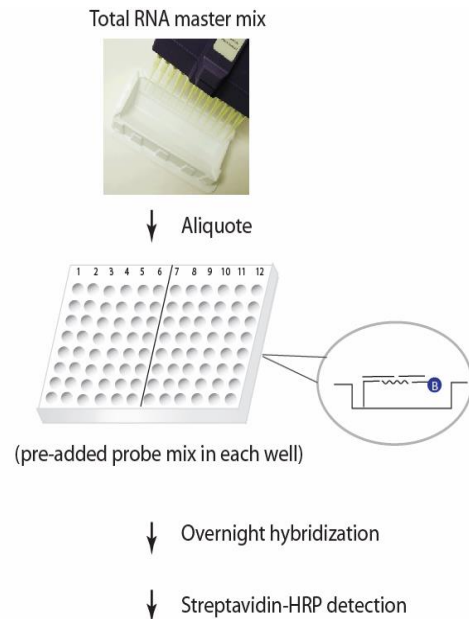
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Introduction

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' stem cell-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.



Chemiluminescent detection with a plate reader

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 dH₂O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH₂O 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 sealing foil securely and incubate the plate at 42 °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.**
5. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200µl of pre-warmed 1x Plate Hybridization Wash Buffer.
6. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
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. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
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

Schematic diagram of Stem Cell-Associated miRNA Plate Array

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