



MiRNA Direct Hybridization Plate Array I

Catalog Number MA-1001

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Introduction

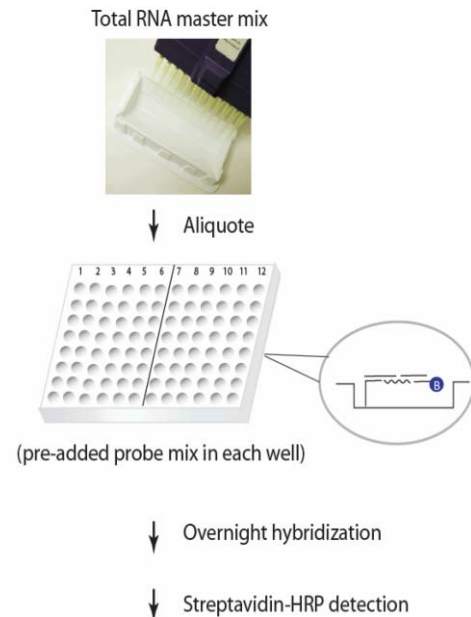
miRNAs are known to involve in multiple biological functions and abnormal expression is associated to human diseases such as cancers. They represent a promising new class of cancer biomarkers. A number of multiplex methods such as microarray or microbeads assays are commonly used for profiling the expression of multiple miRNAs simultaneously. However these methods need the conversion of the miRNA into a cDNA probe during analysis. Signosis has developed a simple miRNA direct hybridization plate array method, in which total RNA does not need any pre-treatment before hybridization. It is as simple as direct hybridization and detection. More importantly, total 48 miRNAs can be quantitatively analyzed and compared simultaneously with the sample assay.

Principle of the assay

Signosis' 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.

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



Chemiluminescent detection with a plate reader

Diagram of miRNA Direct Hybridization Plate Array

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.
- 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 10.

Assay procedure

1. Remove the sealing film from the plate.
2. Mix the following items in a 50 ml reagent reservoir.
 - 5.5ml Hybridization Buffer
 - 10-30 μ l RNA (10-30 μ g)
 - 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 200 μ l 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 200 μ l 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 μ l of diluted streptavidin-HRP conjugate to each well and incubate for 45min 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 95 μ l 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

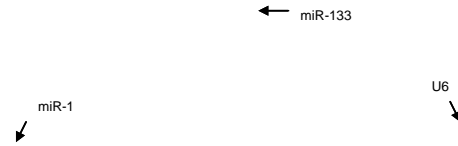


Figure 1: miRNA direct hybridization plate array I analysis of 48 miRNA expression in skeletal muscle and kidney.

Schematic diagram of miRNA Direct Hybridization Plate Array I

let7b	miR-17-5p	miR-29a	miR-132	miR-182	miR-206
miR-1	miR-18a	miR-34a	miR-133	miR-192	miR-210
miR-7	miR-19a	miR-92	miR-143	miR-194	miR-214
miR-9	miR-19b	miR-101a	miR-145	miR-196a	miR-222
miR-10b	miR-20a	miR-106a	miR-146a	miR-199a	miR-223
miR-15a	miR-21	miR-107	miR-148a	miR-200	miR-342
miR-15b	miR-24	miR-122a	miR-155	miR-204	miR-372
miR-16-1	miR-26a	miR-125a	miR-181a	miR-205	U6