



Cancer miRNA Profiling Plate Assay Kit I

Catalog Number MA-0103

(For Research Use Only)

Introduction

miRNAs have been implicated in regulating various biological processes such as cell death and cell proliferation. They can act as oncogenes and tumor suppressor genes, playing a crucial role in tumorigenesis. The change in the expression level of miRNAs is associated with dysfunction of their corresponding protein-coding gene targets, many of which are involved in initiation and progression of cancer. For example, BCL2 oncogene is targeted by miR-15a and miR-16 target, PTEN tumor suppressor by miR-21, HOXD10 by miR-10b, Pak by miR-7, MYOD1 and ER by miR-206, and Her2 by miR-125a. Signosis developed cancer miRNA plate assay kit I for quantitatively profiling the expression of seven well-known cancer related miRNAs, miRNA-15a, miR-16, miR-21, miR10b, miR-7, miR-125a and miR206. U6 is included for normalization.

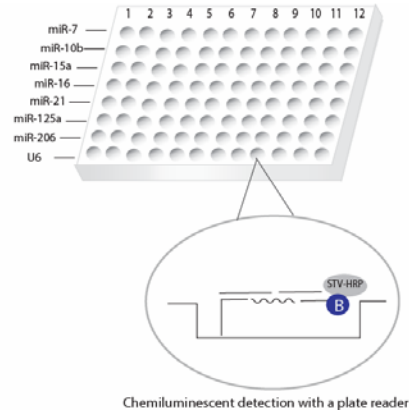


Diagram of miRNA plate array

Principle of the assay

Signosis' proprietary miRNA plate array is a plate-based detection. In the assay, one miRNA molecule is flanked by a capture oligo and a biotinated detection oligo through two bridge oligos. One of the bridge oligos is partially hybridized with the miRNA molecule and the capture oligo and another one with the miRNA and the detection oligo. The hybrid is captured onto plate through hybridization with an immobilized oligo and detected by a streptavidin-HRP conjugate and chemiluminescent substrate. This hybrid structure is sensitive to the sequence of the miRNA molecule. One nucleotide difference can prevent the formation of the hybrid and therefore miRNA isoform can be differentiated, which normally is hard to do with Northern blot. In addition, the sensitivity of the assay is higher than miRNA Northern blot assay.

Materials provided with the kit

- One 96-well white plate (4°C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Block buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)

Material required but not provided

- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection
- ddH₂O (RNAase free)

Reagent preparation before starting experiment

- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45°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 1000 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.
Dilute Biotin Detection Oligo 100 times.
5ul Biotin Detection Oligo + 495ul ddH2O
For each well
2ul -5 µl RNA (0.2µg-2 µg)
100 µl Plate hybridization buffer
4ul Diluted 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, wash the plate by adding 200µl of pre-warmed 1x Plate Hybridization Wash Buffer for 5 min at room temperature with gently shaking. Repeat wash step for 3 times.
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 45min at room temperature with gentle shaking.
10. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate with 200ul 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.
11. Repeat (4) for additional 2 time washes.
12. Freshly prepare the substrate solution
For the whole plate:
1ml Substrate A
1ml Substrate B
8ml Substrate dilution buffer
13. Add 95µl substrate solution to each well and incubate for 1minutes.
14. 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

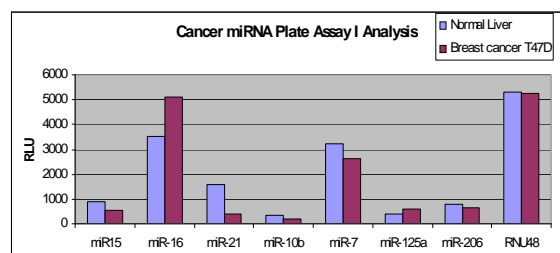


Figure1: Cancer miRNA plate assay analysis of expression of seven genes. Expression of miRNAs was analyzed with 0.5ug total RNA from normal liver and T47D cells respectively. The assay was subjected to chemiluminescent plate reader.

Cancer miRNA plate Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7	miR-7
B	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b	miR-10b
C	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a	miR-15a
D	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16	miR-16
E	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21	miR-21
F	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a	miR-125a
G	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206	miR-206
H	U6	U6	U6	U6	U6	U6	U6	U6	U6	U6	U6	U6