Signosis
Innovative Plate Assay Solutions

MiR-17-92 Cluster Plate Assay Kit

Catalog Number MA-0102 **(For Research Use Only)**

Introduction

A polycistronic miRNA cluster miR-17-92 plays a role in the control of cell proliferation and angiogenesis. This cluster consists of seven miRNAs; miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92. Like E2F1 gene, the cluster is transcriptionally activated by the proto-oncogene c-Myc. Because two of the expressed miRNAs, miR-17-5p and miR-20a, repress translation of E2F1, the level of E2F1 protein is modulated by this feedback mechanism. Therefore, the accumulation of excessive amounts of E2F1 can be prevented and the states of cells towards apoptosis or cell division can be controlled. In addition, c-MYC-mediated induction of the miR-17-92 cluster results in down-regulation of the anti-angiogenic thrombospondin-1 and related proteins, such as connective tissue growth factor.2, and therefore it promotes angiogenesis. Furthermore, a recent study indicates that the cluster is significantly up-regulated at the clonal expansion stage of adipocyte differentiation. The miR-17-92 cluster has been implicated as oncogenes in numerous tumor types. Effective monitoring of the expression of the cluster miRNAs can help us better understand how the alteration of the cluster miRNAs might contribute to development of cancers and shed light on the molecular mechanisms of miRNA function.

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 chemiluminecscent 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.

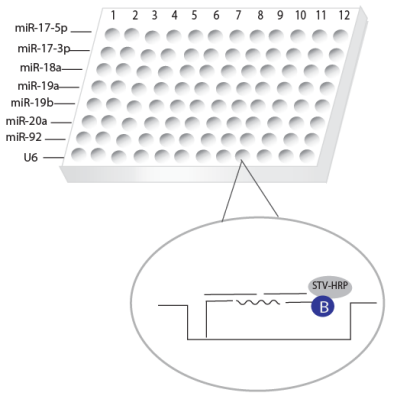

Diagram of miRNA plate array

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 (4°C)
- 8 different miRNA oligo mixes

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 dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute 1000 times of streptavidin-HRP with block buffer before use at Step 7.

Assay procedure

1. Warm up the plate to room temperature, and arrange the appropriate number of the wells of the plate based on your experiment by removing the top foil sealing film with a blade. Keep the unused well sealed.

   Dilute each of the oligo mixes 30X. 1 µl of oligo mix plus 29 µl of RNase free water.

   Mix the following items in one well.
   2 - 5 µl RNA (0.2 - 2 µg)
   100 µl Plate hybridization buffer
   4 µl 1x Oligo mix
   4 µl Biotin Detection Oligo

2. Seal the wells with foil film securely, and hybridize the plate at 42 ^{o}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.

3. Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.

4. Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.

5. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

6. Add 200µl of Blocking buffer to each well and incubate for 15 minutes at room temperature with gently shaking.

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

8. Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.

9. Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.

10. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.

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. Place the plate in the luminometer. Allow plate to sit inside machine for 5 min before reading. 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

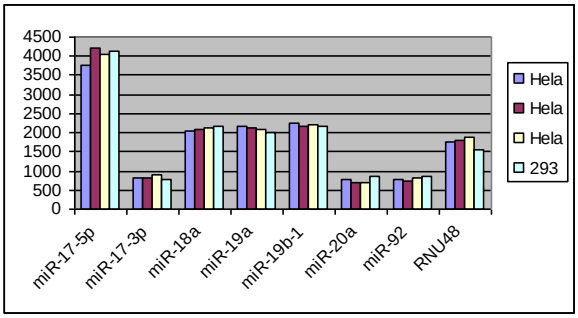

Figure1: miR-17-92 cluster plate analysis of miR-17-92 cluster expression. Expression of miR-17-92 plus U6 was analyzed with 0.5ug total RNA prepared from HeLa cell and 293 cells through miR-17-92 plate assay. Among the assays, HeLa total RNA was analyzed three times.