Signosis
Innovative Plate Assay Solutions

MiRNA Plate Assay Kit

Catalog Number MA-0101 **(For Research Use Only)**

Introduction

MicroRNAs (miRNAs) are small noncoding RNAs that control gene expression at the posttranscriptional level through selective binding to complementary messenger RNA sequences. Approximately 30% of mammalian genes are regulated by these small RNA molecules, which lead to the regulation of various biological functions, including development, cell differentiation, proliferation, apoptosis, and maintenance of stemness and imprinting. Northern blotting has been the most commonly used method for analyzing individual miRNAs, although more recently a number of new approaches have been described, including the related real-time PCR and invader assays for quantifying individual miRNAs. Low sensitivity and poor throughput are the main disadvantages of Northern blot analysis. Signosis' proprietary miRNA plate assay does not need biochemical conversion of miRNA molecules into cDNAs, and the procedure of the assay only requires incubating and washing.

Principle of the assay

Signosis' proprietary miRNA plate array utilizes plate-based detection. In the assay, one miRNA molecule is flanked by a capture oligo and a biotinylated 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 the 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 a Northern blot. In addition, the sensitivity of this assay is higher than a miRNA Northern blot assay.

Materials required but not provided

- miRNA detection oligo mix (MO-XXXX)
- ddH_2O (RNAase free)
- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection

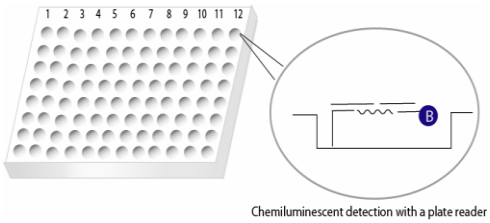

Diagram of miRNA plate array

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 (4°C)
- 5X Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (4°C)

Reagent preparation before starting experiment

- Warm up Plate Hybridization buffer, 5x Plate Hybridization wash buffer, Blocking buffer, and 5X Detection wash buffer at 42°C before use.
- Dilute 30ml of 5X Plate Hybridization wash buffer with 120ml of ddH_2O. Warm up to 42°C before use.
- Dilute 40ml of 5X Detection wash buffer with 160ml of ddH_2O. Warm up to 42°C before use.
- Dilute streptavidin-HRP with Blocking buffer 500 times 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 foil sealing film with a blade. Keep the unused wells sealed.

   Dilute each oligo mix 30 times with RNAase free ddH_2O

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

2. Seal the wells with foil film securely and incubate the plate at 42°C overnight. Ensure the numbers and letters on the plate are clearly visible from under the foil seal by pressing the foil down on every single experimental well.

3. Remove the foil sealing film with a blade to expose the experimental wells. Keep the unused wells sealed for future usage.

4. Invert the plate over an appropriate container and expel the contents forcibly. Then, wash the plate by adding 200µl of warmed 1X Plate Hybridization wash buffer. Completely remove the liquid at each wash by firmly tapping the plate against clean paper towels. Repeat the washing process two times for a total of three washes.

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

6. Invert the plate over an appropriate container to remove the Blocking buffer.

7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 30 minutes at room temperature with gentle shaking.

8. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate with 200µl 1X Detection wash buffer and allow the buffer to incubate in the wells for 5 minutes. Completely remove the liquid at each wash by firmly tapping the plate against clean paper towels. Repeat this step two times for a total of three washes.

9. Prepare the substrate solution:
   For the whole plate:
   1ml Substrate A
   1ml Substrate B
   8ml Substrate dilution buffer

10. Add 95µl substrate solution to each well and incubate for 1 minute.

11. Place the plate in a 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

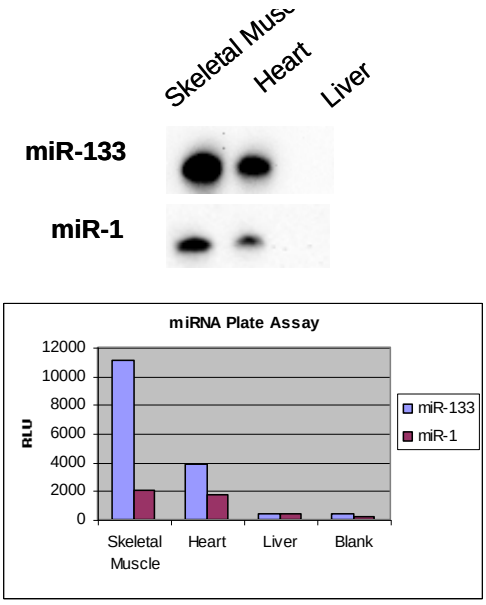

Figure1: miRNA plate analysis of miRNA expression. Expression of miR-133 and miR-1 were analyzed with 5ug total RNA prepared from human skeletal muscle, heart, and liver through miRNA Northern blot (top) and miRNA plate assay (bottom).