Signosis
BioSignal Capture

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 selectively 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 issue 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 is as simple as incubation and wash.

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.

Material required but not provided

- miRNA detection oligo mix (MO-XXXX)
- Hybridization incubator
- Shaker
- ddH2O (RNAase free)
- Plate reader for chemiluminescent detection
  Luminometer with:
  Sensitivity≥ 3 x 10^{-21}moles of luciferase
  Dynamic range ≥ 8 decades
  Well-to-well uniformity < ± 5%

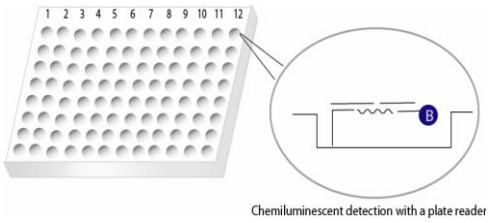

Diagram of miRNA plate array

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 (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)
- Foil film

Reagent preparation before starting experiment

- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 °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 dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute 500 times of streptavidin-HRP with blocking buffer before use at Step 10.

Assay procedure

1. Remove the sealing film. Arrange the appropriate number of the wells of the plate based on your experiment. Seal unused well with foil film (provided).

   Make fresh 30X dilution of oligo mix

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

2. Seal the whole plate 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 foil seal by pressing the foil down on every single experimental well.

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

4. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 200µl 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.

5. Add 200µl of Block buffer incubate for 15 minutes at room temperature with gentle shaking.

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

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

8. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate with 200 µl 1X Detection wash buffer for 5 minutes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. Repeat the wash step for additional 2 times

9. Freshly 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 min.

11. Determine the chemiluminescence of each well with a luminometer and read the plate within 5-20 minutes. For luminescent detection, there is no filter requirement.

Example of Data Analysis

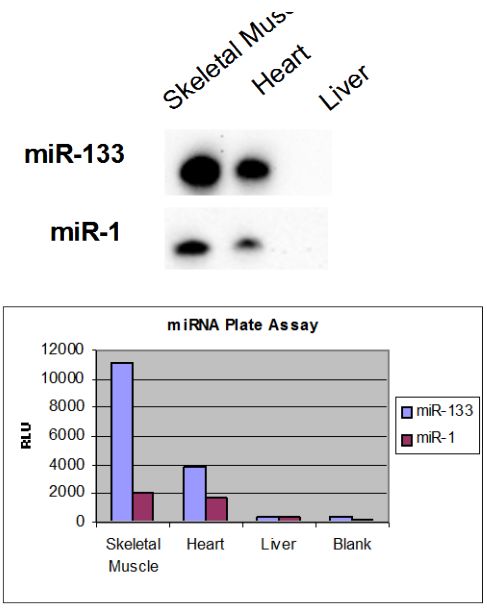

Figure1: miRNA plate analysis of miRNA expression. Expression of miR-133 and miR-1 were analyzed with 5ug total RNA prepared from human skeletal muscle, heart, and liver through miRNA Northern blot (top) and miRNA plate assay (bottom).