Signosis
BioSignal Capture

Stem Cell-Specific MiRNA Plate Assay Kits

Catalog Number MA-0129 **(For Research Use Only)**

Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules, regulating up to 30% of mammalian gene expression. Some of these molecules appear to be stem cell specific. Microarray analysis of miRNA expression displays a set of unique miRNAs in stem cells. Signosis developed a panel of miRNA plate assay kits to analyze these miRNA molecules.

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 bridges oligos. One of the bridge oligos is partially hybridized with the miRNA molecule and the capture oligo and another with the miRNA and the detection oligo. The hybrid is immobilized 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 will prevent the formation of the hybrid and therefore miRNA isoform can be differentiated, which normally is hard to tackle with Northern blot. The captured miRNAs are detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer. The expression level of miRNAs is directly proportional to the chemiluminescent intensity. The sensitivity of the assay is higher than miRNA Northern blot assay.

Materials provided with the kit

- One 96-well plate (4°C)
- A set of miRNA detection oligo mix (MO-XXXX)
- 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)

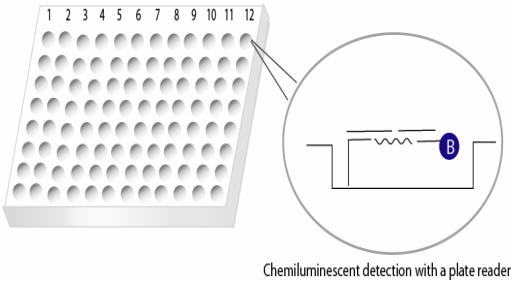

Diagram of miRNA plate array

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 10.

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.

   Mix the following items **freshly** in one well.
   2ul -5 µl RNA (0.2µg-2 µg)
   100 µl Plate hybridization buffer
   4 µl Oligo 1 (miRNA specific)
   4 ul Oligo 2 (miRNA specific)
   4ul Biotin Detection Oligo

2. Seal the wells with foil film securely and incubate the plate at 42 ^{o}C for overnight. Note: put an open container in the incubator, such as empty pipette tip box with water to keep the humidity.

3. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200µl of pre-warmed 1x Plate Hybridization Wash Buffer.

4. 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 block 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. Wash the plate 3 times with 1X Detection wash buffer. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

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 microplate reader within 5 minutes.