Signosis
Innovative Plate Assay Solutions

MiR-21 Plate Assay Kit

Catalog Number MA-0104 **(For Research Use Only)**

Introduction

miR-21 is overexpressed in a wide variety of cancers. It is believed to be an oncogene by downregulating the expression of many tumor suppressors, including programmed cell death 4 (PDCD4), tumor suppressor Pdcd4, the PTEN tumor suppressor gene, and the tumor suppressor gene tropomyosin 1 (TPM1). miR-21 also found to be an important regulator of cancer-cell survival. Differently from the survival pathway mediated by IL-6/Stat3 activation through expression of antiapoptotic proteins, miR-21 is upregulated by Stat3, which mediates the suppression of apoptosis possibly through the inhibition of TPM1 and/or other proteins. Signosis' miR-21 plate assay provides a simple and quick assay to analyze this miRNA molecule.

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.

Materials provided with the kit

- One 96-well plate (4°C)
- miR-21 oligo mix
- 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)

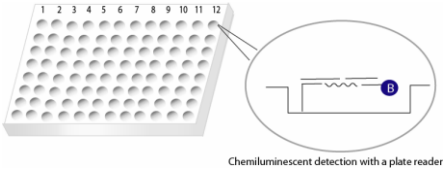

Diagram of miRNA plate array

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.

   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

4. Seal the wells with foil film securely and hybridize at 45 ^{o}C for overnight. Ensure the numbers, letters and edge of each well on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*

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. 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.

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.

13. 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

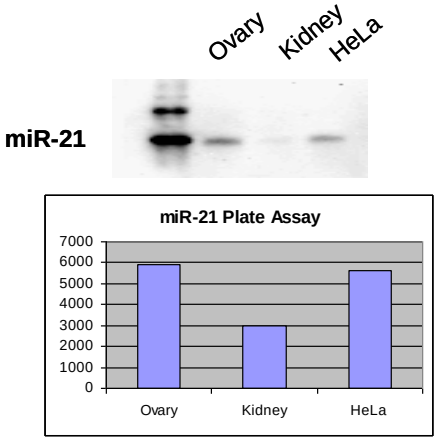

Figure1: miRNA plate analysis of miR-21 expression. Expression of miR-21in human ovary, kidney, and HeLa was analyzed with miR-21 Northern blot (top) and miR-21 plate assay (bottom).