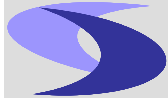

Signosis
Innovative Plate Assay Solutions

SiRNA Plate Assay Kit

Catalog Number MA-0201 **(For Research Use Only)**

Introduction

Small interfering RNAs (siRNAs) are double-stranded RNA molecules with approximately 20-25 nucleotides in length. Since synthetic siRNAs were shown to be able to induce RNAi in mammalian cells in 2001, siRNAs have drawn lots of attention in biomedical research and drug development. They have been widely used for silencing gene expression. To monitor the delivery and expression of siRNA is important to ensuring the success of the knockdown. Northern blot is a common approach to analyze the molecules. However, the assay is not sensitive and the procedure tedious. Signosis has developed a high sensitive siRNA plate assay. It is 1000 times more sensitive than Northern blot. In addition, the assay is just simple incubations, as simple as ELISA. More importantly, a large of samples can be analyzed simultaneously.

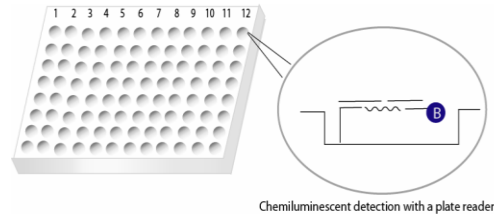

Diagram of siRNA plate array

Principle of the assay

In the proprietary siRNA plate assay, one siRNA 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 siRNA molecule and the capture oligo and another one with the siRNA 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 siRNA molecule. One nucleotide difference can prevent the formation of the hybrid and therefore siRNA isoform can be differentiated, which normally is hard to do with Northern blot. In addition, the sensitivity of the assay is higher than siRNA Northern blot assay.

Materials provided with the kit

- One 96-well plate (RT)
- Streptavidin-HRP conjugate (4^oC)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Block buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4^oC)
- Substrate B (4^oC)
- Substrate dilution buffer (RT)
- Oligo mix

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 ^oC before use.
- Dilute 30ml of 5x Plate Hybridization wash buffer with 120 ml of dH_2O before use. Stir the solution with a 10ml or 5ml pipette to facilitate the dissolving process.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute 500 times of streptavidin-HRP with block buffer before use at Step 10.

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Assay procedure

1. 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

2. Seal the wells with foil film securely and incubate the plate at 50 ^{o}C overnight.

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. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate with 200ul 1X Detection wash buffer for 5 min at room temperature with gently shaking. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

9. Repeat (8) for additional 2 time washes.

10. Freshly prepare the substrate solution
    For the whole plate:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer

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

12. 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

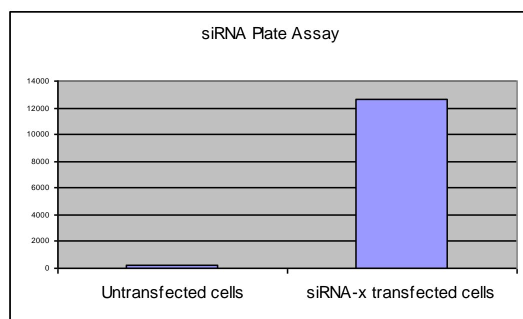

Figure1: siRNA plate analysis. Expression of siRNA-x was analyzed with 5ug of total RNA prepared from siRNA-x transfected HeLa cells.