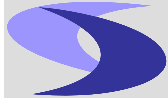

Signosis
BioSignal Capture

Quantitative mRNA Plate Assay Kit

Catalog Number RA-1001 **(For Research Use Only)**

Introduction

Gene expression is commonly measured through a number of steps including RNA preparation, reverse transcription, and PCR. To simplify the analysis of gene expression, Signosis has developed a multiple-biotin signal amplification (MBSA) technology. Based on this technology, gene expression can be directly quantitatively measured directly in cell lysates without RNA preparation, reverse transcription and PCR. Signosis has developed the assay kits for quantitatively measuring TNF and IL-6. Now, we offer service for developing quantitative mRNA plate assay for any genes. Just provide us gene information such as Gene ID and we provide you the assay kit.

Principle of the assay

In MBSA, a polymer containing multiple biotins binds to the targeted RNA via hybridization of a linker. The linker contains two moieties, one binds to the polymer and another to the targeted molecule. A set of linkers can be designed, which shares a common tag sequence, although they contain different target sequences to hybridize different regions of the targeted molecule. Therefore, it leads to the binding of multiple biotin polymers to multiple regions. The captured biotin molecule is last detected with streptavidin-HRP and a chemiluminescent substrate.

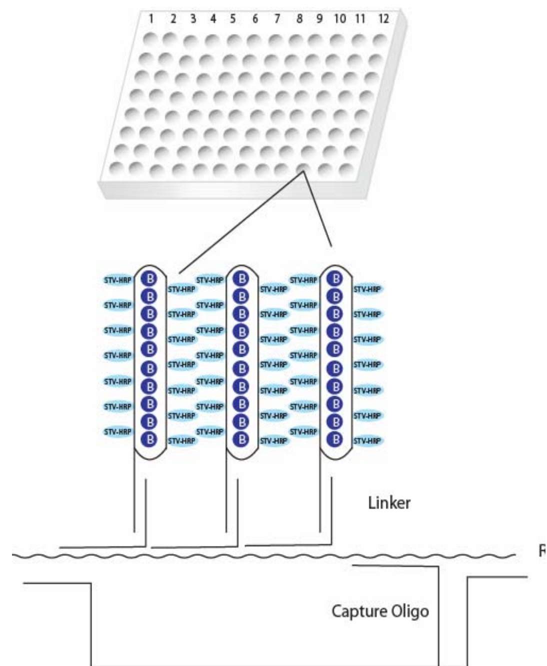

Diagram of mRNA Quantitative Assay

Materials provided with the kit

- One 96-well plate (RT)
- IVT RNA standard (-20°C)
- Target gene oligo mix (-20°C)
- GAPDH Oligo Mix (-20°C)
- Multiple-biotin molecules (-20°C)
- MBSA hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Streptavidin-HRP conjugate (4°C)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)
- Sealing foil

Instrument and materials required

- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection

Reagent preparation before starting experiment

- Warm up Plate hybridization buffer and Hybridization Wash buffer at 42°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 blocking buffer before use at Step 10.

Assay procedure

1. Arrange the appropriate number of the wells of the plate based on your experiment. Removing the top foil sealing film with a blade to expose the experimental wells. Make sure the rest of wells are well sealed.

2. Mix the following items in each well:
   100ul MBSA Hybridization Buffer
   1-5 µl total RNA (0.2~1 µg)
   2ul Target Gene Oligo Mix
   For the internal control well, add 2 ul GAPDH Oligo Mix instead of target gene oligo mix.

3. Cut new sealing foil (provided) to fit the area of the experimental wells. Seal the wells with sealing foil (provided) securely. 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. Incubate the plate at 50 ^{o}C overnight.

4. Remove the foil from the top of the assay wells. 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.

5. Add 100ul MBSA Hybridization Buffer and 4ul Multiple-biotin molecules, seal the wells with sealing foil securely and incubate the plate at 50 ^{o}C for 1 hour.

6. Remove the foil from the top of the assay wells. 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.

7. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

8. Add 200µl of Blocking buffer and incubate for 15 minutes at room temperature with gentle shaking.

9. Invert the plate over an appropriate container to remove block buffer.

10. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45min at room temperature with gentle shaking.

11. 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.

12. Repeat (11) for additional 2 time washes.

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

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

15. 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.

Making a standard curve

1. Make a master mix with 1ml plate hybridization buffer and 20ul target gene Oligo Mix.
2. Transfer 110ul to the first well, and 100ul to the rest of 7 wells.
3. Add 2ul target gene IVT to the first well, mix well.
4. Transfer 10ul to the following well.
5. Repeat the step 4 to make 10X serial dilution.
6. Keep the eighth well for the blank.

Example of Data Analysis

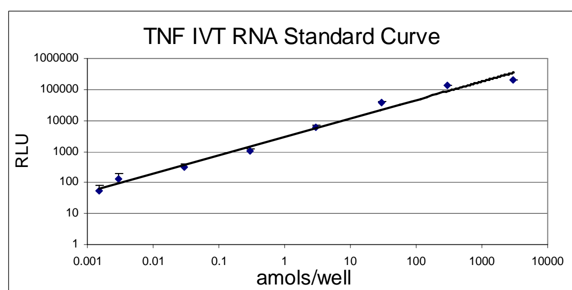

Figure1: TNF IVT RNA Standard Curve. TNF IVT RNA was serially diluted and subjected to TNF quantitative RNA plate assay. The signal is measured with a luminescent plate reader.