



## Human TNF Quantitative mRNA Plate Assay

Catalog Number RA-0001

(For Research Use Only)

### Introduction

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is one of inflammatory cytokines. It plays a variety of functions, although many of them are not yet fully understood. It is produced by different types of cells, but especially by macrophage. Altered expression of inflammatory cytokines including TNF- $\alpha$  has been shown to associate with different diseases, such as inflammatory bowel disease. Monitoring the expression of these cytokines is therefore a common approach to dissect the molecular mechanism underlying the diseases. In addition, the emergence of molecular medicine has resulted in the desire of quantitative analysis of these molecules in clinical diagnostics. Signosis has developed a multiple-biotin signal amplification (MBSA) technology. It is 1000 times more sensitive than single biotin-based detection. Based on this technology, Signosis developed TNF- $\alpha$  quantitative mRNA plate assay. It can absolutely measure human TNF- $\alpha$  mRNA in a lower femogram range.

### 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.

### Instrument and materials required

- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection

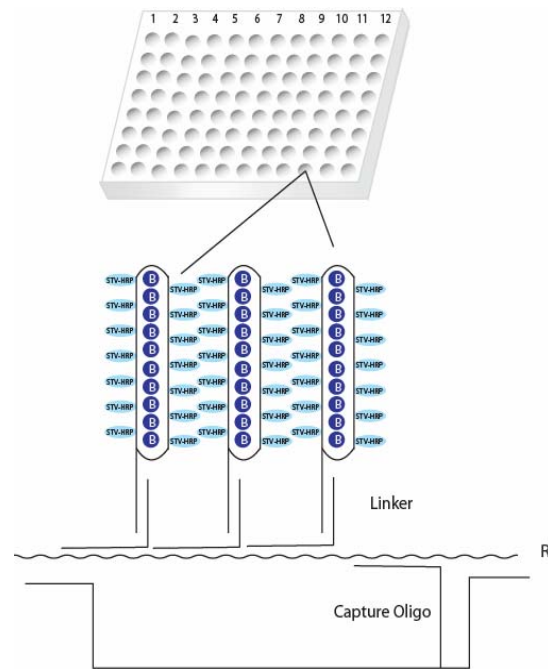


Diagram of mRNA Quantitative Assay

### Materials provided with the kit

- One 96-well plate (RT)
- Human TNF IVT RNA standard (-20°C)
- Human TNF oligo mix (-20°C)
- Human 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

## 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<sub>2</sub>O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH<sub>2</sub>O 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 Human TNF Oligo Mix  
For the internal control well, add 2 ul GAPDH Oligo Mix instead of TNF 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 °C overnight. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*
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 °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 human TNF Oligo Mix.
2. Transfer 110ul to the first well, and 100ul to the rest of 7 wells.
3. Add 2ul human TNF 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

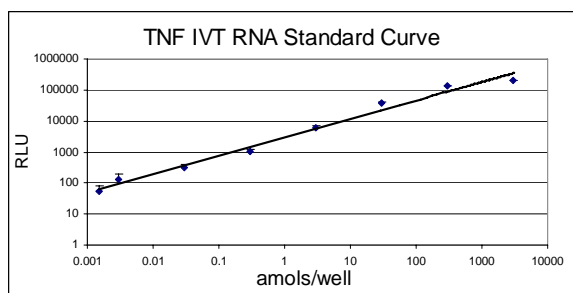


Figure1: TNF IVT RNA Standard Curve. TNF IVT RNA was serially diluted and subjected to TNF quantitative RNA plate assay. The signal is measured with a luminescent plate reader.

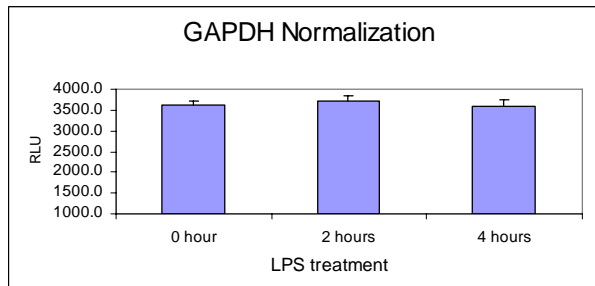
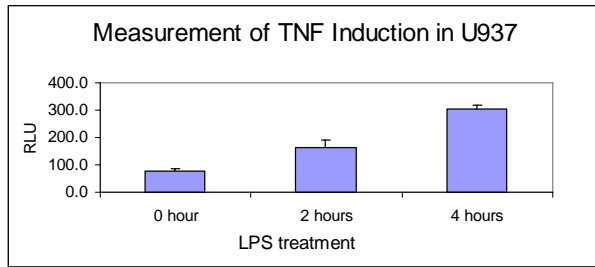


Figure 2. Measurement of TNF induction in U937. U937 cells were treated with and without 50ng/ml LPS for 2 and 4 hours. 1ug of total RNA was used for TNF quantitative mRNA plate assay for TNF expression. GAPDH is used for normalization.