



Human IL6 Quantitative mRNA Plate Assay

Catalog Number RA-0002

(For Research Use Only)

Introduction

Interleukin 6 (IL-6), which was originally identified as a B-cell differentiation factor, is now known to be a multifunctional cytokine that regulates the immune response, hematopoiesis, the acute phase response, and inflammation. It is produced by activated T cells, macrophages, endothelial cells, fibroblasts and osteoblasts. Deregulation of IL-6 production is implicated in the pathology of several disease processes, such as autoimmune diseases and chronic inflammatory proliferative diseases. Monitoring the expression of this cytokine is therefore a common approach to dissect the molecular mechanism underlying the diseases. 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 IL6 quantitative mRNA plate assay, which can be used for quantitative measurement of IL6 gene expression directly in cell lysates as well in total RNA.

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

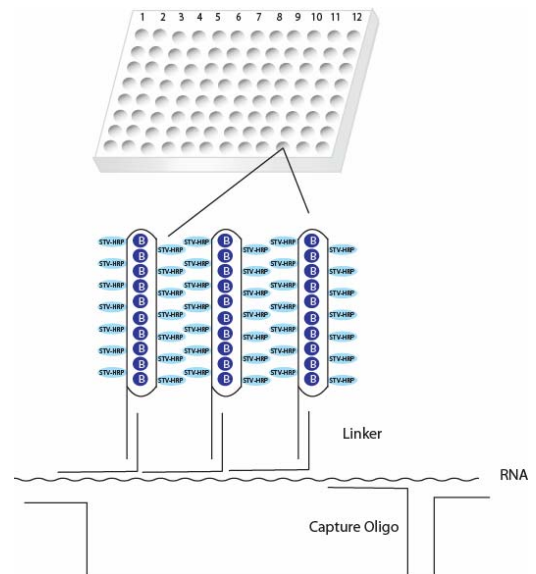


Diagram of mRNA Quantitative Assay

Materials provided with the kit

- One 96-well plate (RT)
- Human IL6 IVT RNA standard (-20°C)
- Human IL6 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)

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 dH₂O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH₂O 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. Sample Preparation:

a. Starting material from total RNA

The total RNA can be prepared with many methods, such as Trizol reagent and RNA preparation columns.

Mix the following items in each well (the master mix can be prepared depending on the number of the samples)

- 100ul MBSA Hybridization Buffer
- 1-5 µl total RNA (0.2-1 µg)
- 2ul IL6 Oligo Mix

For the internal control well, add 2 ul GAPDH Oligo Mix instead of IL6 oligo mix.

b. Starting material from cell lysate

The cells are cultured on the wells of a 96-well plate (1-210⁴ cells/well). 100ul cell lysis buffer add on the each well, pipette up and down and incubate at 37°C for 3 hours. The cell lysate can be used directly for the assay or stored at -80 °C for the future usage.

Mix the following items in each well (the master mix can be prepared by multiplying the following volume of Plate Hybridization Buffer and Oligo Mix with the number of the samples):

- 80ul Plate Hybridization Buffer
- 20 µl cell lysate
- 2ul IL6 Oligo Mix

For the internal control well, add 2 ul GAPDH Oligo Mix instead of IL6 oligo mix.

3. Cut the sealing foil (provided), fitting the area of the experimental wells. Seal the wells with the sealing foil securely. Ensure the wells well sealed through examining the numbers and letters on the plate underneath the foil seal, which should be clearly visible. Incubate the plate at 50 °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. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

5. Add 100ul Plate Hybridization Buffer and 4ul Multiple-biotin molecules to each well. Seal the wells with the 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. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

7. 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 IL6 Oligo Mix.
2. Transfer 110ul to the first well, and 100ul to the rest of 7 wells.
3. Add 2ul human IL6 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 blank

Example of standard curve

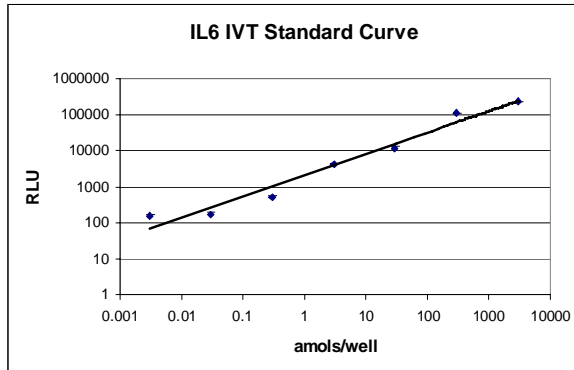


Figure1: IL6 IVT RNA Standard Curve. IL6 IVT RNA was serially diluted and subjected to IL6 quantitative RNA plate assay. The signal is measured with a luminescent plate reader.