Signosis
BioSignal Capture

Human Inflammation ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1031 **(For Research Use Only)**

Introduction

Cytokines are extracellular signalling proteins produced by different cell types that act on target cells to modulate diverse cellular functions, such as recruiting specific cell types to the site of inflammation, increasing the activation and survival of immune cells, or suppressing cellular activity. Inflammation is the response of tissue to injury. During both acute and chronic inflammatory processes, a variety of soluble factors are involved in the cellular infiltrate, the cellular activation, and the systemic responses to inflammation. Cytokines are major determinants of inflammatory responses. Most cytokines are multifunctional molecules that elicit their effects locally or systemically in an autocrine or paracrine manner. Cytokines are involved in extensive networks that involve synergistic as well as antagonistic interactions and exhibit both negative and positive regulatory effects on various target cells. Therefore, profiling the expression pattern of cytokines provides a valuable insight to the underlying immunological mechanisms. Signosis' Inflammation ELISA Strip allows quantitatively profiling and measuring 8 cytokines; TNFa, IFNr, G-CSF, GM-CSF, IL-1a, IL-8, IP-10, and Rantes.

Principle of the assay

In each well of the strip, a primary antibody against a specific inflammation cytokine is coated and 8 wells of the strip are coated with 8 different antibodies. Therefore, total 8 wells of a strip allow measurement of 8 different cytokines. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the inflammation cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentrations of the inflammation cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

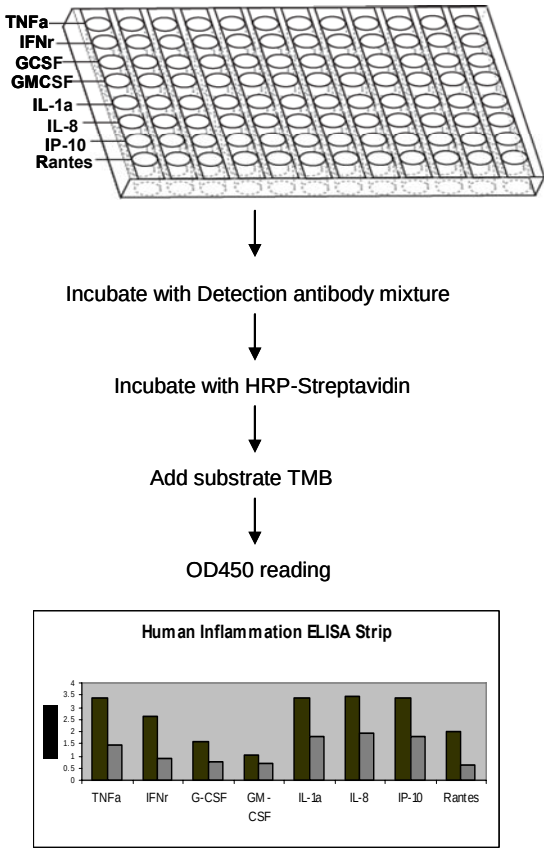


Diagram of Human Inflammation ELISA Strip

Materials provided with the kit

- 12 strips, each coated with 8 different antibodies against human inflammation cytokines (4°C).
- Biotin labeled antibody mixture against 8 different inflammation cytokines (-20°C).
- Streptavidin-HRP conjugate (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (RT)
- Substrate (4°C)
- Stop Solution (4°C)

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

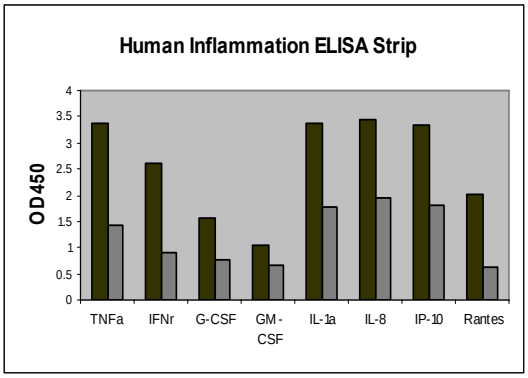
Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the inflammation cytokines among the samples can be easily identified and determined.
- If you would like to quantitatively measure the cytokines in the samples, please order EA-1032. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Add 100 µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl substrate to each well and incubate for 5-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve



	Black bar	Grey bar
TNFα	2000pg/ml	500pg/ml
IFNr	2000pg/ml	500pg/ml
G-CSF	4000pg/ml	1000pg/ml
GM-CSF	4000pg/ml	1000pg/ml
IL-1a	2000pg/ml	500pg/ml
IL-8	2000pg/ml	500pg/ml
IP-10	2000pg/ml	500pg/ml
Rantes	4000pg/ml	1000pg/ml