



## Human Cytokine ELISA Strip I for Profiling 8 Cytokines

Catalog Number EA-1081

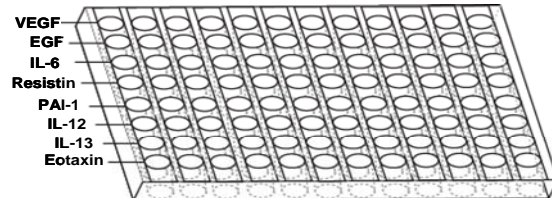
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### Introduction

Cytokines are essential molecules that play crucial roles in many biological functions, including viral infection, inflammation, immunity, and hematopoiesis. Cytokines are produced by a variety of cell types in response to different stimuli. In addition, the expression of cytokine genes appears to be regulated by complex mechanisms. Expression of one cytokine gene could be regulated by other cytokines. Dysregulation of cytokine gene expression may be caused by chromosomal alterations or by infection of viruses that induce activation or inactivation of the expression machinery. Therefore, profiling of these cytokines is critical to understanding these biological functions. Signosis' Human Cytokine ELISA Strip I Profiling Assay allows simultaneous profiling of 8 cytokines; VEGF, EGF, IL-6, Resistin, PAI-1, IL-12, IL-13 and Eotaxin. Each well of the strip is coated with a primary antibody against a specific cytokine and total 8 wells of a strip target 8 different cytokines. The difference of these proteins between two samples can be determined through data comparison.

### Principle of the assay

In each well of the strip, a primary antibody against a specific angiogenesis 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 angiogenesis 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 angiogenesis cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Incubate with Detection antibody mixture

Incubate with HRP-Streptavidin

Add substrate TMB

OD450 reading

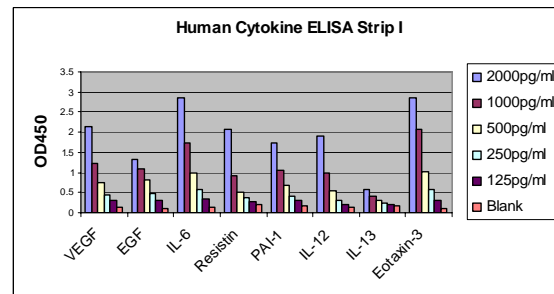


Diagram of Human Cytokine ELISA Strip I

### Materials provided with the kit

- 12 strips, each coated with 8 different antibodies against human cytokines (4°C).
- Biotin labeled antibody mixture II against 8 different human 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 ddH<sub>2</sub>O
- 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 II 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 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-1082. 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  $\mu$ l 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 200 $\mu$ l 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 100 $\mu$ l of diluted biotin-labeled human cytokine antibody mixture I 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  $\mu$ l 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 100 $\mu$ l substrate to each well and incubate for 5-30 minutes.
9. Add 50 $\mu$ l 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

