**Signosis**
Innovative Plate Assay Solutions

# Human Interleukin ELISA Strip for Profiling 8 Cytokines

**Catalog Number EA-1311** **(For Research Use Only)**

## Introduction

Interleukins are a family of chemical messengers that can evoke a wide spectrum of responses in the body, including immune and inflammatory responses. Understanding the conditions that alter their expression can be critical for unraveling the underlying mechanisms of diseases such as Rheumatoid Arthritis, Atherosclerosis, and a variety of cancers. Signosisõs Interleukin ELISA Strip Profiling Assay can simultaneously analyze 8 interleukins, including IL1α, IL2, IL-4, IL6, IL8, IL10, IL-12, and IL-13.

## Principle of the assay

Each well of the strip is coated with a specific capture antibody to detect its corresponding cytokine in the sample. Therefore, 8 different proteins can be measures simultaneously. The test sample reacts simultaneously with two antibodies, resulting in the cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. The HRP substrate, TMB, is then added and causes a blue color change. The reaction is then terminated with Stop Solution, resulting in a yellow color. The concentrations of oxidative stress cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm. The expression levels of these cytokines can be quantitatively compared between samples.

## Materials provided with the kit

- 12 strips, each coated with 8 different antibodies against interleukin cytokines (4°C).
- Biotin-labeled antibody mixture against 8 different interleukin 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)

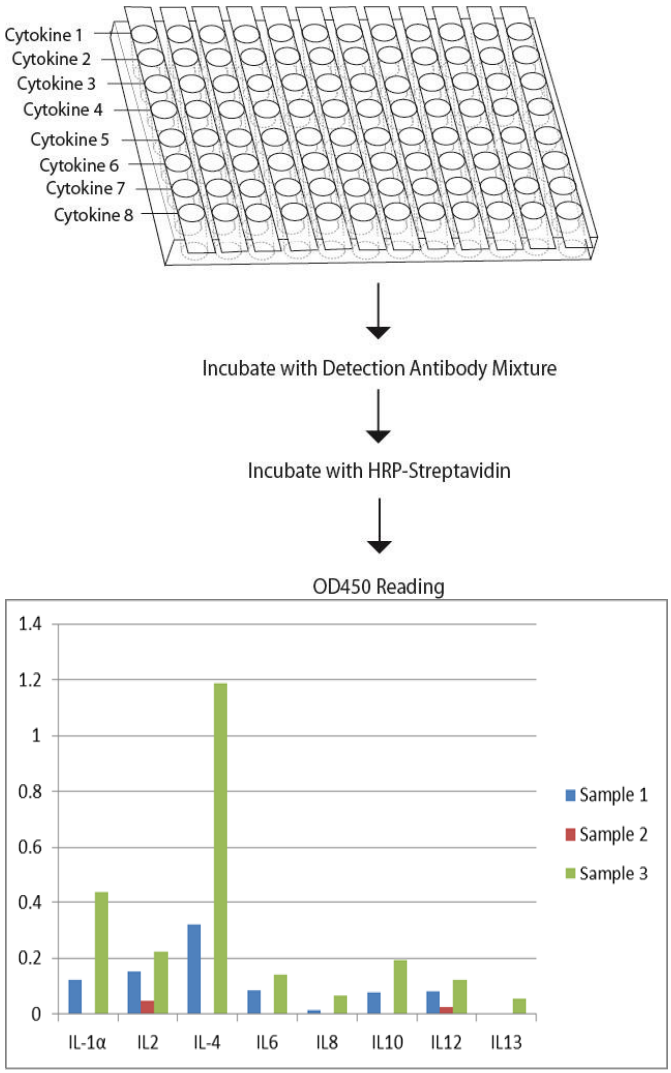


Diagram of Human Interleukin ELISA Strip Analysis

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer:
  40ml 5x Assay wash buffer
  160ml ddH2O
- To dilute standards, refer to Standards User Manual.
- Dilute biotin-labeled antibody mixture 1:50 with 1X Diluent Buffer.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer.

## Sample preparation before starting experiment

- For **media samples**, add 100εl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer

## Recommendations

- The product is intended to be used for comparisons between 2-12 different samples. This can be done without the use of external standards.
- If you would like to quantitatively measure the proteins in the samples, please order EA-1312, a complete set of protein standards for this assay, which can be used to make 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 remain 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 and completely removing the liquid. Repeat the process three times for a total of three washes. 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 10-30 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.

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α | IL-1α |
| 2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 | IL-2 |
| 3 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 | IL-4 |
| 4 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 | IL-6 |
| 5 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 | IL-8 |
| 6 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 | IL-10 |
| 7 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 | IL-12 |
| 8 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 | IL-13 |