**Signosis**
Innovative Plate Assay Solutions

# Human Cytokine ELISA Plate Array I (Colorimetric)

**Catalog Number EA-4002** **(For Research Use Only)**

## Introduction

Cytokines are signaling molecules that have critical roles in many biological processes such as cellular growth, differentiation, gene expression, migration, immunity, and inflammation. Cytokines are secreted from cells and bound to cell-surface receptors, which initiate the activation of signal transduction pathways and mediate cell to cell communication. The malfunction of cytokines leads to many diseases including arthritis, acute and chronic liver disease, inflammatory bowel disease, cardiac-related diseases, and cancers. A group of cytokines commonly involves in one biological or disease process, therefore, the comprehensive analysis of the expression of multiple cytokines allows revealing the underneath mechanism of the disease state effectively. The Human Cytokine ELISA Plate Array I allows you to monitor the abundance of 32 human cytokines in a high-throughput manner. This assay is a fast and sensitive tool for quantitatively profiling the levels of multiple cytokines between samples simultaneously.

## Principle of the assay

The 96-well clear plate is divided into 3 sections, and each section has 4 strips for one sample. In each section, 32 of specific cytokine capture antibodies are coated on 32 wells respectively. The sample such as cell culture supernatants, cell lysates, tissue homogenates, serum, or plasma samples is incubated with cytokine ELISA plate, and the captured cytokine proteins are subsequently detected with a cocktail of biotinylated detection antibodies. The test sample is allowed to react with pairs of 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. 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 human cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

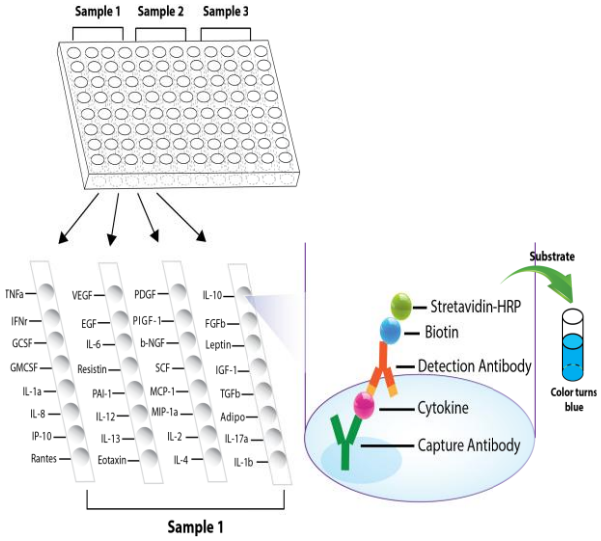


Diagram of Human Cytokine ELISA Plate Array Assay

## Materials provided with the kit

| Component | Qty | Store at |
|---|---|---|
| **96-Well Plate coated with 32 different antibodies against Human Cytokines** | 1 | 4 °C |
| **32 Biotin-labeled anti-human detection antibody mix** | 200 µL | -20 °C |
| **Streptavidin-HRP conjugate** | 50 µL | 4 °C |
| **1xDiluent buffer** | 40 mL | 4 °C |
| **5X Assay wash buffer** | 40 mL | 4 °C |
| **Substrate** | 10 mL | 4 °C |
| **Stop solution** | 5 mL | 4 °C |

## Material required but not provided

- Microplate reader
- Distilled H2O

## Reagent preparation before starting experiment

- Dilute the 5X Assay wash buffer to 1X buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
  (AVOID FREEZE/THAW OF ANTIBODY MIX)
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

## Sample preparation before starting experiment

- For **cell culture medium samples**, add 100 ul directly to the well or dilute 2-fold with 1X Diluent buffer.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual on our website.
- For **serum or plasma samples**, we recommend a 1:10 to 1:20 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

## Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare 3.5 ml sample and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
   **Optional:** If you want to have a blank reading, you can design one well as a blank well by adding diluent buffer instead of your sample.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200 µl of 1X Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100 µl of diluted biotin-labeled antibody mixture to each well and incubate for 2 hours at room temperature with gentle shaking. (For lower concentration samples, the plate can be incubated overnight at 4°C for a stronger signal).
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 100 µl substrate to each well and incubate for 30 to 40 minutes at least.

   **Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10 to 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 Analysis Data**

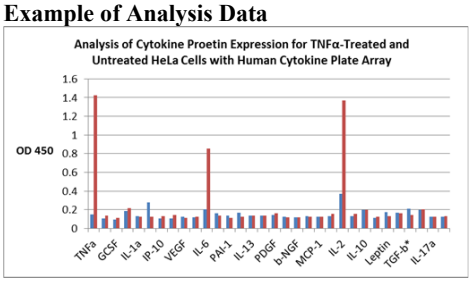


Figure1: Analysis of Cytokine Protein Expression in TNFα-Treated and Untreated HeLa with Human Cytokine ELISA Plate Array
HeLa cells were starved for 24 hours with serum-free medium, subsequently treated the cells with and without 20 ng/ul TNFα for 16 hours. The serum-free conditioned media were incubated on the plate for 1 hour. After incubating with detection antibody mix and HRP, the plate was detected by a plate reader.

**Diagram of Human Cytokine ELISA Plate Array I**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TNFα | VEGF | PDGF-BB | IL-10 | TNFα | VEGF | PDGF-BB | IL-10 | TNFα | VEGF | PDGF-BB | IL-10 |
| B | IFNγ | EGF | PIGF-1 | FGFb | IFNγ | EGF | PIGF-1 | FGFb | IFNγ | EGF | PIGF-1 | FGFb |
| C | GCSF | IL-6 | β-NGF | Leptin | GCSF | IL-6 | β-NGF | Leptin | GCSF | IL-6 | β-NGF | Leptin |
| D | GM-CSF | Resistin | SCF | IGF-1 | GM-CSF | Resistin | SCF | IGF-1 | GM-CSF | Resistin | SCF | IGF-1 |
| E | IL-1α | PAI-1 | MCP-1 | TGF-b | IL-1α | PAI-1 | MCP-1 | TGF-b | IL-1α | PAI-1 | MCP-1 | TGF-b |
| F | IL-8 | IL-12 | MIP-1α | Adipo | IL-8 | IL-12 | MIP-1α | Adipo | IL-8 | IL-12 | MIP-1α | Adipo |
| G | IP-10 | IL-13 | IL-2 | IL-17A | IP-10 | IL-13 | IL-2 | IL-17A | IP-10 | IL-13 | IL-2 | IL-17A |
| H | Rantes | Eotaxin | IL-4 | IL-1β | Rantes | Eotaxin | IL-4 | IL-1β | Rantes | Eotaxin | IL-4 | IL-1β |