Signosis
BioSignal Capture

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 31 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 columns for one sample. In each section, 31 of specific cytokine capture antibodies are coated on 31 wells respectively, and one well without coating any antibody is used as a blank well. The sample, such as cell culture supernatants, cell lysates, tissue homogenates, serum, or plasma samples is incubated with cytokine ELISA plate, 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 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

- One clear plate coated with 31 different antibodies against human cytokines (4°C).
- Biotin labeled antibody mixture against 31 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)

Material required but not provided

- Microplate reader
- Distilled H2O

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Sample preparation
  - Serum-free cell culture conditioned media can be used directly or dilute 2-fold with 1X Diluent buffer before use. When serum-containing conditioned media is required, be sure to use serum as a control.
  - Cell lysate or tissue lysate can be prepared following the protocol on our website: http://www.signosisinc.com/pdf/Preparation_of_Cell_Lysates_for_ELISA.pdf
  - Sera or plasma can be used directly or diluted 2-10 fold with 1X Diluent buffer.
- Dilute 100 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Cut the film over the plate and remove it from the desired columns. Make sure the rest of plate is well sealed.
2. Prepare 3.5ml sample and add 100 µl of sample per well to one section and incubate for 2 hours at room temperature with gentle shaking.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200µl 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 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 30 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 30 minutes at least.
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 40 minutes.

Example of Analysis Data

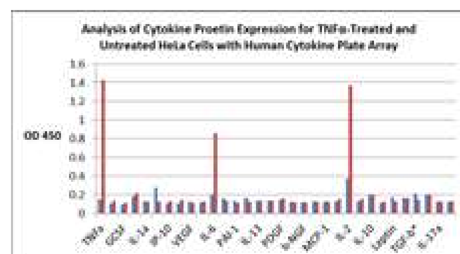

Figure1: Analysis of Cytokine Protein Expression in TNFα-Treated and Untreated HeLa with Human Cytokine ELISA Plate Array

HeLa cells were starved for 24 hours with serum-free medium, subsequently treated the cells with and without 20ng/ul TNF 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	TNFa	VEGF	PDGF	IL-10	TNFa	VEGF	PDGF	IL-10	TNFa	VEGF	PDGF	IL-10
B	IFNr	EGF	PLGF	FGFb	IFNr	EGF	PLGF	FGFb	IFNr	EGF	PLGF	FGFb
C	GCSF	IL-6	b-NGF	Leptin	GCSF	IL-6	b-NGF	Leptin	GCSF	IL-6	b-NGF	Leptin
D	GMCSF	Resistin	SCF	IGF-1	GMCSF	Resistin	SCF	IGF-1	GMCSF	Resistin	SCF	IGF-1
E	IL-1a	PAI-1	MCP-1	TGF-beta	IL-1a	PAI-1	MCP-1	TGF-beta	IL-1a	PAI-1	MCP-1	TGF-beta
F	IL-8	IL-12	MIP-1a	Adipo	IL-8	IL-12	MIP-1a	Adipo	IL-8	IL-12	MIP-1a	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	Blank	Rantes	Eotaxin	IL-4	Blank	Rantes	Eotaxin	IL-4	Blank