Signosis
Innovative Plate Assay Solutions

Human Cytokine ELISA Plate Array IV (Chemiluminescence)

Catalog Number EA-4020 **(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 that are secreted from cells bind to cell-surface receptors, 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. Cytokines are commonly working together in a biological or disease process. Therefore, the comprehensive analysis of the expression of multiple cytokines allows effective revealing of the underneath mechanism of cytokine action and the alteration leading to diseases. The Human Cytokine ELISA Plate Array is a chemiluminescent detection that allows you to monitor the abundance of 48 human cytokines simultaneously. This fast and sensitive assay can be used for quantitative comparison of these cytokines among different samples.

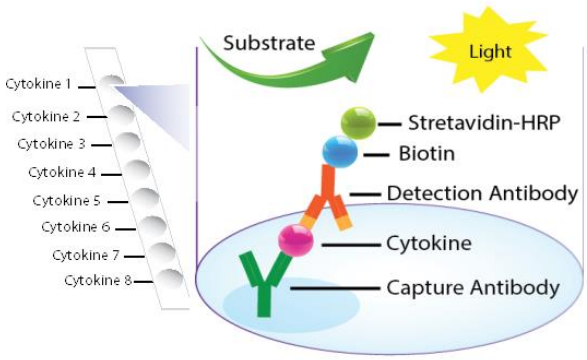

Diagram of Human Cytokine ELISA Plate Array Assay

Principle of the assay

The 96-well white plate is divided into 2 sections, and each section has 6 strips for one sample. In each section, 48 of specific cytokine capture antibodies are coated on 48 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, 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. The plate is further detected with HRP luminescent substrate. Luminescence is reported as relative light units (RLUs) on a microplate luminometer. The level of expression for each specific cytokine is directly proportional to the luminescent intensity.

Materials provided with the kit

Component	Qty	Store at
96-Well white Plate coated with 48 different antibodies	1	4°C
48 Biotin-labeled anti-human detection antibody mix	50 µL	-20°C
Streptavidin-HRP conjugate	10 µL	4°C
1xDiluent buffer	40 mL	4°C
5X Assay wash buffer	40 mL	4°C
Substrate A	1 mL	4°C
Substrate B	1 mL	4°C
Substrate dilution buffer	8 mL	4°C

Material required but not provided

- Luminometer plate reader
- Distilled H2O

Reagent preparation before starting experiment

- Dilute the 5X Assay wash buffer to 1X
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Dilute 200 times of biotin labeled antibody mixture with 1X Diluent buffer.
  (AVOID FREEZE/THAW OF ANTIBODY MIX)
- Dilute 1000 times of streptavidin-HRP with 1X Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100ul 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 5 ml sample and add 100 µl per well to one section. Cover the plate and incubate for **2 hour** at room temperature with gentle shaking.
   Optional: If you want to have a blank reading, you can designate 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 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 30 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 3 with an additional wash. Total of 4 washes. ***It is important to add another wash to reduce high background.**
9. Freshly prepare the substrate solution
   For the whole plate:
   1 ml Substrate A
   1 ml Substrate B
   8 ml Substrate dilution buffer
10. Add 95 µl substrate solution to each well and incubate for 2 minutes.
11. Place the plate in the luminometer. Set integration time to 1 second with no filter position and read immediately.

Diagram of Human Cytokine ELISA Plate Array IV

	1	2	3	4	5	6	7	8	9	10	11	12
A	Adipo	CXCL16	IGF	IL-6	IL-22	PDGF-BB	Adipo	CXCL16	IGF	IL-6	IL-22	PDGF-BB
B	b-NGF	EGF	IGF-BP1	IL-8	IL-31	PIGF-1	b-NGF	EGF	IGF-BP1	IL-8	IL-31	PIGF-1
C	CCL27	Eotaxin-3	IL-1a	IL-10	IP-10	Rantes	CCL27	Eotaxin-3	IL-1a	IL-10	IP-10	Rantes
D	CTGF	FGFb	IL-1b	IL-11	Leptin	Resistin	CTGF	FGFb	IL-1b	IL-11	Leptin	Resistin
E	CXCL1	G-CSF	IL-2	IL-12	MCP-1	SCF	CXCL1	G-CSF	IL-2	IL-12	MCP-1	SCF
F	CXCL2	GM-CSF	IL-3	IL-13	MIP-1a	TGFb	CXCL2	GM-CSF	IL-3	IL-13	MIP-1a	TGFb
G	CXCL9	ICAM-1	IL-4	IL-17a	Neuroserpin	TNFa	CXCL9	ICAM-1	IL-4	IL-17a	Neuroserpin	TNFa
H	CXCL11	IFNr	IL-5	IL-17E	PAI-1	VEGF	CXCL11	IFNr	IL-5	IL-17E	PAI-1	VEGF