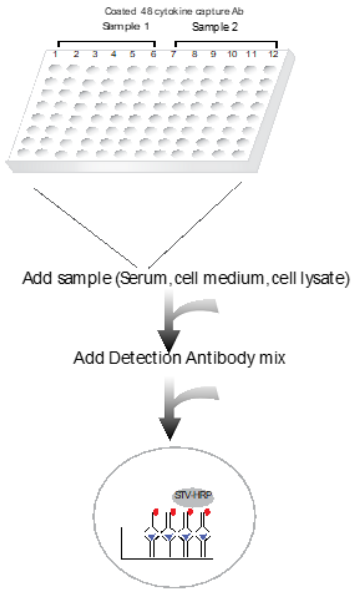
Signosis
Innovative Plate Assay Solutions

Mouse Cytokine ELISA Plate Array IV (Colorimetric)

Catalog Number EA-4014 **(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 Mouse Cytokine ELISA Plate Array IV allows you to monitor the abundance of 46 mouse 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 2 sections, and each section has 6 columes for one sample. In each section, 46 of specific cytokine capture antibodies are coated on 46 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 cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kit

Component	Qty	Store at
One clear plate coated with 46 different antibodies against Mouse Cytokines	1	4°C
Biotin-labeled anti-mouse detection antibody mix	200µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1x Diluent buffer	40mL	4°C
5x Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	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
- 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 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 3.5ml sample in 1x Diluent buffer 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 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 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. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
9. Add 100µl substrate to each well and incubate for 30-40 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 10-30 minutes. Always stop the reaction of samples from the same row at the same time.
10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Diagram of Mouse Cytokine ELISA Plate Array IV

	1	2	3	4	5	6	7	8	9	10	11	12
A	**β-NGF**	**FGFb**	**IL-1RA**	**IL-13**	**Leptin**	**Restistin**	β-NGF	FGFb	IL-1RA	IL-13	Leptin	Restistin
B	**BMP-2**	**G-CSF**	**IL-2**	**IL-15**	**MCP-1**	**SCF**	BMP-2	G-CSF	IL-2	IL-15	MCP-1	SCF
C	**CCL11**	**GM-CSF**	**IL-3**	**IL-17A**	**MCSF**	**TGFβ**	CCL11	GM-CSF	IL-3	IL-17A	MCSF	TGFβ
D	**CCL21**	**IFNγ**	**IL-4**	**IL-21**	**MIP-1α**	**TNFα**	CCL21	IFNγ	IL-4	IL-21	MIP-1α	TNFα
E	**CNTF**	**IGF-1**	**IL-5**	**IL-22**	**MIP-1β**	**TPO**	CNTF	IGF-1	IL-5	IL-22	MIP-1β	TPO
F	**CXCL1**	**IGF-BP1**	**IL-6**	**IL-23**	**Neuroserpin**	**VEGF**	CXCL1	IGF-BP1	IL-6	IL-23	Neuroserpin	VEGF
G	**CXCL10**	**IL-1α**	**IL-10**	**IL-33**	**PDGF-BB**	**Blank**	CXCL10	IL-1α	IL-10	IL-33	PDGF-BB	Blank
H	**EGF**	**IL-1β**	**IL-12**	**IP-10**	**Rantes**	**Blank**	EGF	IL-1β	IL-12	IP-10	Rantes	Blank