Signosis
Innovative Plate Assay Solutions

Mouse Cytokine ELISA Plate Array II (Chemiluminescence)

Catalog Number EA-4021 **(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 II allows you to monitor the abundance of 32 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.

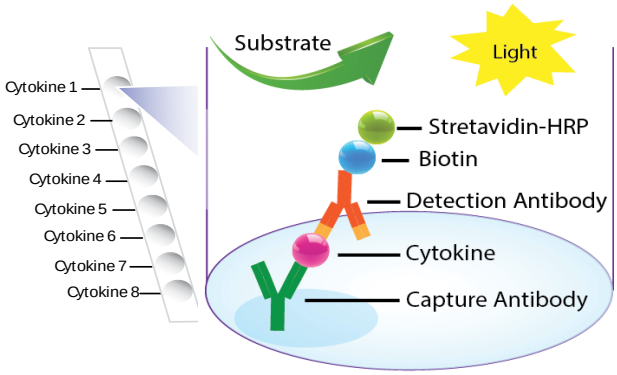

Diagram of Mouse Cytokine ELISA Plate Array Assay

Principle of the assay

The 96-well white 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. 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 32 different antibodies against mouse cytokines	1	4°C
Biotin-labeled anti-mouse 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. Use diluted 3.5ml sample and add 100 µl 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 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.
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 with an additional wash. Total 4 washes. ***It is impartant to include an additional wash to prevent high background***
8. Freshly prepare the substrate solution as following:
   For the whole plate:
   1 mL Substrate A
   1 mL Substrate B
   8 mL Substrate Dilution Buffer
9. Add 95µl substrate solution to each well and incubate for 2 minutes.
10. Place the plate in the luminometer. Set integration time to 1 second with no filter position and read immediately.

Example of Analysis Data

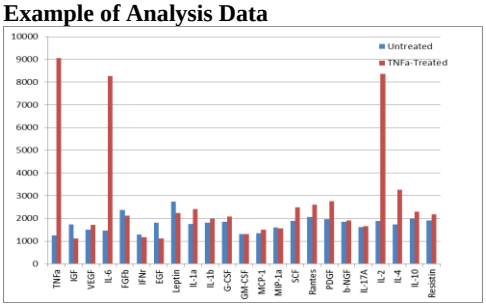

Figure1: Analysis of Cytokine Protein Expression in TNFα-Treated and Untreated HeLa with Mouse Cytokine ELISA Plate Array
NIH3T3 cells were starved for 24 hours with serum-free medium, subsequently treated the cells with and without 20ng/ul TNFα for 16 hours. The serum-free conditioned media were incubated on the plate for 1 hour. After incubating with detection antibody mix and HRP, the plate was detected with chemilumincent substrate by a plate reader.

Diagram of Mouse Cytokine ELISA Plate Array II

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNFα	IL-1α	PDGF-BB	IL-5	TNFα	IL-1α	PDGF-BB	IL-5	TNFα	IL-1α	PDGF-BB	IL-5
B	IGF-1	IL-1β	β-NGF	IL-15	IGF-1	IL-1β	β-NGF	IL-15	IGF-1	IL-1β	β-NGF	IL-15
C	VEGF	G-CSF	IL-17A	IL-23	VEGF	G-CSF	IL-17A	IL-23	VEGF	G-CSF	IL-17A	IL-23
D	IL-6	GM-CSF	IL-2	TPO	IL-6	GM-CSF	IL-2	TPO	IL-6	GM-CSF	IL-2	TPO
E	FGFb	MCP-1	IL-4	IP-10	FGFb	MCP-1	IL-4	IP-10	FGFb	MCP-1	IL-4	IP-10
F	IFNγ	MIP-1α	IL-10	CCL11	IFNγ	MIP-1α	IL-10	CCL11	IFNγ	MIP-1α	IL-10	CCL11
G	EGF	SCF	Resistin	IL-33	EGF	SCF	Resistin	IL-33	EGF	SCF	Resistin	IL-33
H	Leptin	Rangtes	IL-12	CCL21	Leptin	Rangtes	IL-12	CCL21	Leptin	Rangtes	IL-12	CCL21