Signosis
Innovative Plate Assay Solutions

Rat Cytokine ELISA Plate Array I (Chemiluminescence)

Catalog Number EA-4004 **(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 Rat Cytokine ELISA Plate Array I allows you to monitor the abundance of 16 rat 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.

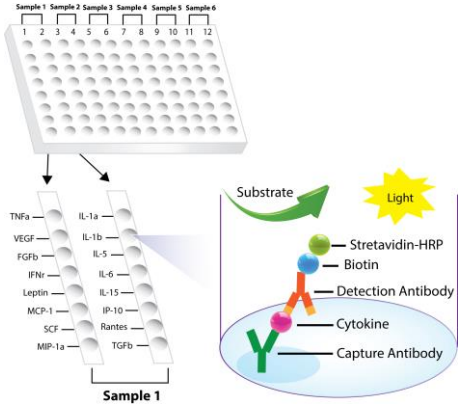

Diagram of Rat Cytokine ELISA Plate Array Assay

Principle of the assay

The 96-well white plate is divided into 6 sections, and each section has 2 strips for one sample. In each section, 16 of specific cytokine capture antibodies are coated on 16 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
96well white Plate coated with 16 different antibodies against Rat Cytokines	1	4°C
16 Biotin-labeled anti-rat detection antibody mix	50µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate A	1mL	4°C
Substrate B	1mL	4°C
Substrate dilution buffer	8mL	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 200 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. Use diluted 1.7 ml sample and add 100 µl per well to one section. Cover the plate 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 200µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Completely remove 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 45 min at room temperature with gentle shaking.
7. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200µl of 1x Assay wash buffer. Incubate wash buffer for 10 minutes on a shaker. Repeat washing process two times for a total of three washes with 10 minutes incubation between each wash. Completely remove liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
   Note: It is important to incubate wash buffer for 10 minutes during each wash to reduce high background in the blank wells.
8. Freshly prepare the substrate solution
   For the whole plate:
   1ml Substrate A
   1ml Substrate B
   8ml Substrate dilution buffer
9. Add 95µl substrate solution to each well and **incubate for 2 minutes**.
10. Place the plate in the luminometer, and read. Set integration time to 1 second with no filter position. For the best results, read the plate **immediately** .

Diagram of Rat Cytokine ELISA Plate Array I

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNFa	IL-1a	TNFa	IL-1a	TNFa	IL-1a	TNFa	IL-1a	TNFa	IL-1a	TNFa	IL-1a
B	VEGF	IL-1b	VEGF	IL-1b	VEGF	IL-1b	VEGF	IL-1b	VEGF	IL-1b	VEGF	IL-1b
C	FGFb	IL-5	FGFb	IL-5	FGFb	IL-5	FGFb	IL-5	FGFb	IL-5	FGFb	IL-5
D	IFNy	IL-6	IFNy	IL-6	IFNy	IL-6	IFNr	IL-6	IFNy	IL-6	IFNy	IL-6
E	Leptin	IL-15	Leptin	IL-15	Leptin	IL-15	Leptin	IL-15	Leptin	IL-15	Leptin	IL-15
F	MCP-1	IP-10	MCP-1	IP-10	MCP-1	IP-10	MCP-1	IP-10	MCP-1	IP-10	MCP-1	IP-10
G	SCF	Rantes	SCF	Rantes	SCF	Rantes	SCF	Rantes	SCF	Rantes	SCF	Rantes
H	MIP-1a	TGFb	MIP-1a	TGFb	MIP-1a	TGFb	MIP-1a	TGFb	MIP-1a	TGFb	MIP-1a	TGFb