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 15 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.

Principle of the assay

The 96-well white plate is divided into 6 sections, and each section has 2 columns for one sample. In each section, 15 of specific cytokine capture antibodies are coated on 15 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.

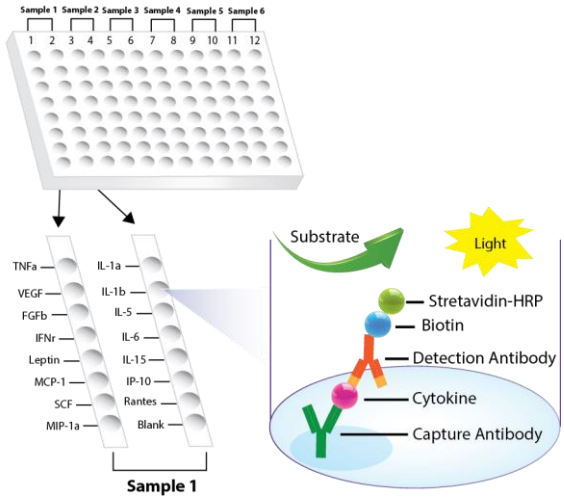

Diagram of Rat Cytokine ELISA plate array assay

Materials provided with the kit

- One clear plate coated with 15 different antibodies against rat cytokines (4°C)
- Biotin labeled antibody mixture against 15 different rat cytokines (-20°C)
- Streptavidin-HRP conjugate (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (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 diluted 10-20 fold with 1X Diluent buffer.
- Dilute 200 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 number of well strips. Make sure the rest of wells are well sealed.
2. Use diluted 1.7 ml sample and add 100 µl 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. 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. Complete removal of 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 within 5-20 minutes.

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	IFNr	IL-6	IFNr	IL-6	IFNr	IL-6	IFNr	IL-6	IFNr	IL-6	IFNr	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	Blank	MIP-1a	Blank	MIP-1a	Blank	MIP-1a	Blank	MIP-1a	Blank	MIP-1a	Blank