Signosis
Innovative Plate Assay Solutions

Mouse Inflammation ELISA Strip (Chemiluminescence)

Catalog Number EA-1711 **(For Research Use Only)**

Introduction

Cytokines are extracellular signaling proteins produced by different cell types that act on target cells to modulate diverse cellular functions, such as recruiting specific cell types to the site of inflammation, increasing the activation and survival of immune cells, or suppressing cellular activity. Inflammation is the response of tissue to injury. During both acute and chronic inflammatory processes, a variety of soluble factors are involved in the cellular infiltrate, the cellular activation, and the systemic responses to inflammation. Cytokines are major determinants of inflammatory responses. Most cytokines are multifunctional molecules that elicit their effects locally or systemically in an autocrine or paracrine manner. Cytokines are involved in extensive networks that involve synergistic as well as antagonistic interactions and exhibit both negative and positive regulatory effects on various target cells. Therefore, profiling the expression pattern of cytokines provides a valuable insight to the underlying immunological mechanisms. Signosis' Inflammation ELISA Strip Assay quantitatively profiles and measures 8 cytokines; IL-1α, IL-1β, G-CSF, GM-CSF, MCP-1, MIP-1α, SCF, and Rantes. The difference of these proteins between two samples can be determined through data comparison.

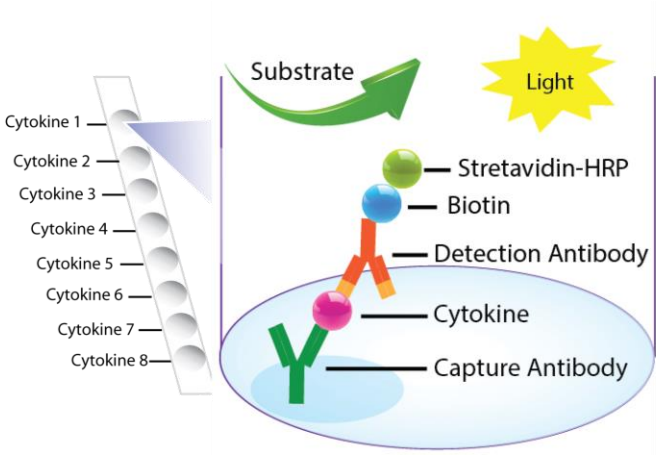

Diagram of Mouse Inflammation ELISA Strip

Principle of the assay

In each well of the strip, a primary antibody against a specific cytokine is coated and each of the 8 wells of the strip is coated different antibodies. Therefore, each strip can measure of 8 different proteins. The test sample is allowed to react simultaneously 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 is directly proportional to the luminescent intensity.

Component	Qty	Store at
96-Well 12 strip Plate coated with 8 different antibodies against mouse inflammation cytokines	1	4°C
Biotin-labeled antibody mixture against 8 different mouse inflammation cytokines	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1x Diluent buffer	40 mL	4°C
5x Assay wash buffer	40 mL	4°C
Substrate dilution buffer	8 mL	4°C
Substrate A	1 mL	4°C
Substrate B	1 mL	4°C

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Refer to Standards User Manuel for diluting standards.
- Dilute 50 times of biotin labeled antibody mixture with 1x Diluent buffer.
- Dilute 1000 times of streptavidin-HRP with 1x Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1x Diluent buffer, for example, add 80µl sample in 720µl 1x Diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the cytokines among the samples can be easily identified and determined.
- If you would like to quantitatively measure the cytokines in the samples, please order EA-1052. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Follow EA-1052 user manual)

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Standard curve:
If protein standard curve is desired, 4-5 strips may be used to make Standard curve (Please see the user manual for EA-1052 for detail).
3. Sample assay:
Apply each sample in one strip, 100µl per well and incubate for 1-2 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200µl of 1x Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 3.
7. Add 100µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Freshly prepare the substrate solution.
For whole plate:
1 ml Substrate A
1 ml Substrate B
8 ml Substrate dilution buffer
9. Invert the plate over an appropriate container and expel the contents forcibly, then firmly tap the plate against clean paper towels. 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.

Note: It is important to incubate wash buffer for 10 minutes during each wash to reduce background.

10. Add 95µl 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**.

Mouse Inflammation ELISA Strip (Chemiluminescence) Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α
B	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
C	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF	G-CSF
D	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
E	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
F	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α
G	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF
H	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes