Signosis
Innovative Plate Assay Solutions

Human Neuroinflmmation ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1121 **(For Research Use Only)**

Introduction

Neuroinflammatory process has a fundamental role in the pathogenesis of neurodegenerative diseases including Parkinson's, Alzheimer's (AD), ALS, and Huntington's. Cytokines play a key role in inflammatory and anti-inflammatory processes in neurodegenerative diseases. Increasing amounts of evidence indicates that cytokines related inflammatory processes are involved in the neurotoxicity. Therefore, profiling the expression pattern of cytokines provides a valuable insight to the underlying immunological mechanisms. Signosis' Human NeuroInflammation ELISA Strip Assay quantitatively profiles and measures 8 associated cytokines. The list of cytokines is as follows: TNFα, IL-1β, IL1α, IL-13, IL-10, Il-4, IL-6 and TGFβ. The difference of these proteins between two samples can be determined through data comparison.

Principle of the assay

Each well of the strip is coated with a specific capture antibody to detect its corresponding neuroinflammation cytokine in the sample. Therefore, 8 different proteins can be measures simultaneously. The test sample reacts simultaneously with pairs of two antibodies, resulting in the human neuroinflammation cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. The HRP substrate, TMB, is added to result in the development of a blue color. The reaction is then terminated with the addition of Stop Solution, resulting in a yellow color. The concentrations of oxidative stress cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm. The expression levels of these cytokines can be quantitatively compared between samples.

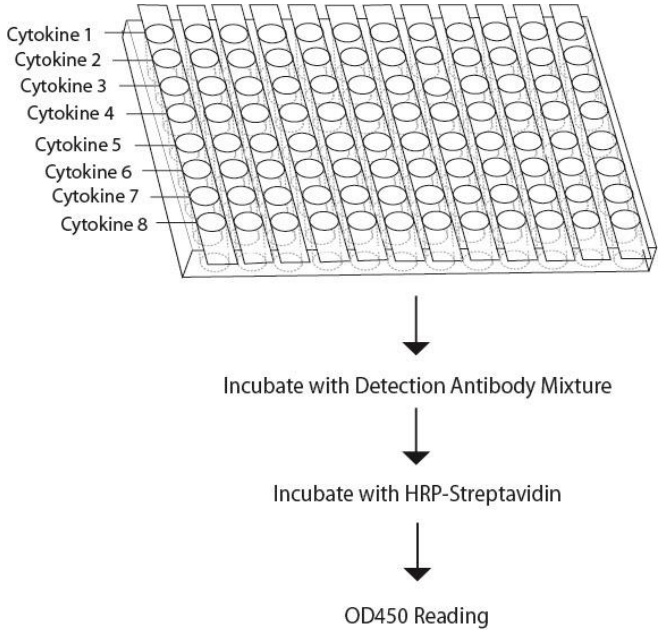

Diagram of Oxidative Stress ELISA Strip Analysis

Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with 8 different antibodies against human neuroinflammation cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human neuroinflammation cytokines	200µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	RT

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40ml 5x Assay wash buffer
  - 160ml ddH_2O.
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 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 80ul sample in 720ul 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 human neuroinflammation 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-1122. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Following EA-1122 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-1122 for detail).
3. Sample assay:
Apply each sample in one strip, 100ul 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 4.
7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100µl substrate to each well and incubate for 10-30 minutes.

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.

Human Neuroinflammation ELISA Strip Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα
B	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α
C	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
D	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4
E	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
F	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10
G	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13	IL-13
H	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ