



## Human Oxidative Stress ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1301

(For Research Use Only)

### Introduction

Oxidative stress can directly damage cell structures and may lead to cancer, inflammatory diseases, and aging. Interestingly, oxidative stress can also alter the expression levels of a variety of cytokines. Understanding the conditions that alter the expression of these cellular messengers is important for unraveling the mechanisms of these diseases and developing therapeutics. Signosis' Human Oxidative Stress ELISA Strip Profiling Assay simultaneously analyzes 8 cytokines; TNF $\alpha$ , TGF $\beta$ , MCP-1, IL-1 $\alpha$ , IL-2, IL-6, IL-10, and IL-12.

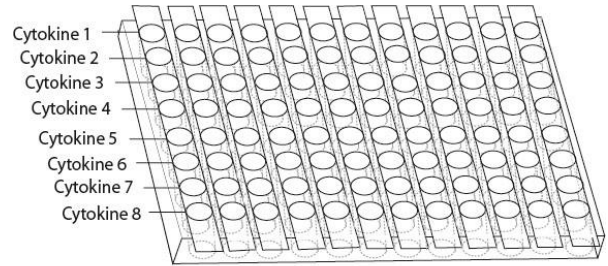
The difference of these proteins between two samples can be determined through data comparison.

### Principle of the assay

Each well of the strip is coated with a specific capture antibody to detect its corresponding cytokine in the sample. Therefore, 8 different proteins can be measured simultaneously. The test sample reacts 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 HRP substrate, TMB, is then added and causes a blue color change. The reaction is then terminated with 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.

### Materials provided with the kit

Component	Qty	Store at
12 strips, each coated with 8 different antibodies against human oxidative stress cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human oxidative stress cytokines	200 $\mu$ L	-20°C
Streptavidin-HRP conjugate	50 $\mu$ L	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	RT



Incubate with Detection Antibody Mixture

Incubate with HRP-Streptavidin

OD450 Reading

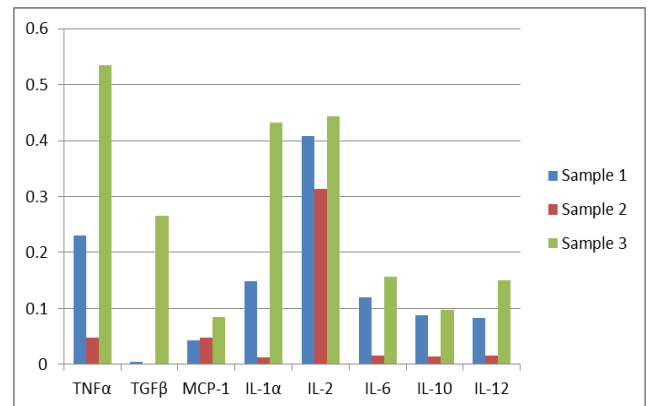


Diagram of Human Oxidative Stress ELISA Strip Analysis

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer:
  - 40ml 5x Assay wash buffer
  - 160ml ddH<sub>2</sub>O
- To dilute standards, refer to Standards User Manual.
- 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 is intended to be used for comparisons between 2-12 different samples. This can be done without the use of external standards.
- If you would like to quantitatively measure the proteins in the samples, please order EA-1302, a complete set of protein standards for this assay, which can be used to make standard curves through a series of 2-fold dilutions.  
(Following EA-1302 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-1302 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 Oxidative Stress ELISA Strip Diagram

	A	B	C	D	E	F	G	H	I	J	K	L
1	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα	TNFα
2	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ
3	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
4	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α
5	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2
6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
7	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10
8	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12