Signosis
Innovative Plate Assay Solutions

Human ER Stress ELISA Strip (Chemiluminescence)

Catalog Number EA-1694 **(For Research Use Only)**

Introduction

The Unfolded Protein Response (UPR) is a conserved and essential stress response that cells activate to combat endoplasmic reticulum (ER) stress, commonly caused by the accumulation of misfolded proteins or failing protein quality control. UPR signaling has been identified as one of the avenues leading to the inflammatory response. The integration of ER stress, oxidative stress and the inflammatory response is critical to the pathogenesis of a variety of diseases, such as such as diabetes, Alzheimer's, Parkinson's, and obesity. It has been demonstrated that some cytokines can activate ER Stress and that ER stress can increase the expression of certain cytokines as well. Therefore, cytokine profiling provides a valuable tool for examining the relationship between cytokine expression and ER stress response. Signosis' Human ER stress ELISA Strip quantitatively profiles and measures 8 ER stress-associated cytokines; TNFα, IL-1β, IFNγ, IL-6 ,IGF-1, MCP-1, Leptin and TGFβ. The difference of these proteins between two samples can be determined through data comparison.

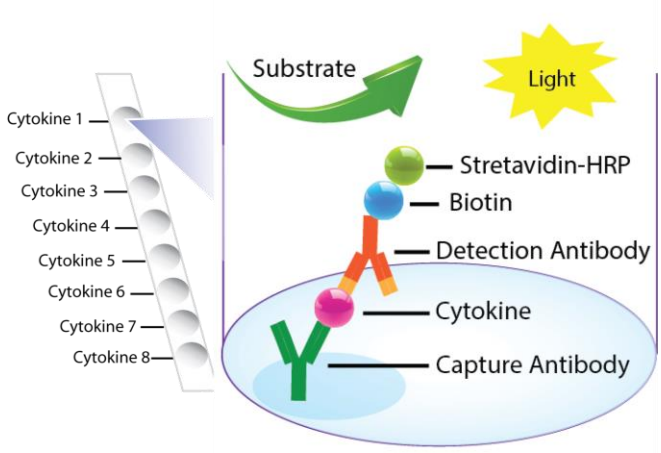

Diagram of Human ER Stress ELISA Strips (Chemiluminescence)

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.

Materials provided with the kit

Component	Qty	Store at
96-Well white 12 strip Plate coated with 8 different antibodies against Human ER Stress cytokines	1	4°C
Biotin-labeled antibody mixture against 8 different Human ER Stress cytokines	200µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate A and B	1mLeach	4°C
Substrate dilution buffer	8mL	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 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 cytokines among the samples can be easily identified and determined.
- If you would like to quantitatively measure the proteins in the samples, please order the corresponding standards, which can be used for making standard curves through a series of 2-fold dilutions.

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.
Note: Add Standards according to the diagram.
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. 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 200 ul 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 incubaition between each wash.

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

10. Add 95 µl 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**.

Diagram of Human ER Stress ELISA Strip

	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-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
C	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ	IFNγ
D	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
E	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1
F	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
G	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin
H	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ