Signosis
Innovative Plate Assay Solutions

Human Th17 Pathway ELISA Strip (Colorimetric)

Catalog Number EA-1331 **(For Research Use Only)**

Introduction

The Th17 pathway is an immune signaling and differentiation pathway involving T helper 17 (Th17) cells, which are a subset of CD4+ T cells. These cells play a crucial role in host defense against extracellular pathogens and are also implicated in the pathogenesis of autoimmune diseases. Cytokines, such as IL-6, IL-1β, and TGFβ, play an important role in regulating the differentiation of Th17 cells. Once differentiated, Th17 cells produce important effector cytokines to recruit and activate neutrophils. These cytokines include IL-17A, IL-22, and GM-CSF. Signosis' Human Th17 Inflammation ELISA Strip Kit quantitatively profiles and measures 8 cytokines which are involved in Th17 pathway. The list of cytokines is as follows: TNFα, IL-1β, IL-6, TGFβ, IL-10, IL-17A, IL-22, and GM-CSF.

Principle of the assay

In each well of the strip, a primary antibody against a specific cytokine is coated and 8 wells of the strip are coated with 8 different antibodies. Therefore, total 8 wells of a strip allow measurement of 8 different cytokines. 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. 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.

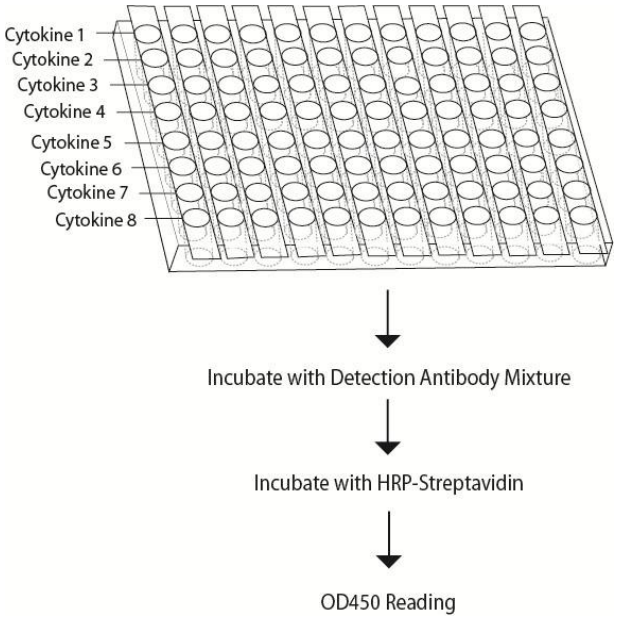

Diagram of Human Th17 Pathway ELISA

Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with 8 different antibodies against human Th17 pathway cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human Th17 pathway 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	10 mL	4°C
Stop solution	5 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 .
- Dilute 50 times of biotin labeled antibody mixture with 1x Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1x Diluent buffer.
- Avoid contact of Substrate and Stop Solution with sunlight or any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

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-1332. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Follow EA-1332 user manual)

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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-1332 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 4.
7. Add 100µl 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. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
10. 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.**

11. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Human Th17 Pathway 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-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
C	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
D	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ	TGFβ
E	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10
F	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A	IL-17A
G	IL-22	IL-2	IL-22	IL-22	IL-22	IL-22	IL-22	IL-22	IL-22	IL-22	IL-22	IL-22
H	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF