



Human Interleukin ELISA Plate Array

Catalog Number EA-4011

(For Research Use Only)

Introduction

Interleukins are cytokines produced and secreted mainly by T lymphocytes. Interleukins promote development and differentiation of natural killer cells, T and B lymphocytes and haematopoietic stem cells. Interleukins are involved in systemic inflammation and immune system modulation, so play important roles in fighting cancer, infectious disease and other diseases. Signosis have newly launched Human Interleukin ELISA Plate Array to study 21 cytokines for 4 samples in one single assay. All of regents are included in the kit.

Principle of the assay

The 96-well clear plate is divided into 4 sections, and each section has 3 strips for one sample. In each section, 21 of specific cytokine capture antibodies are coated on 21 wells respectively. The sample, such as cell culture supernatants, cell lysates, tissue homogenates, serum, or plasma samples is incubated with cytokine ELISA plate, and the captured cytokine proteins are subsequently detected with a cocktail of biotinylated detection antibodies. The test sample is allowed to react 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.

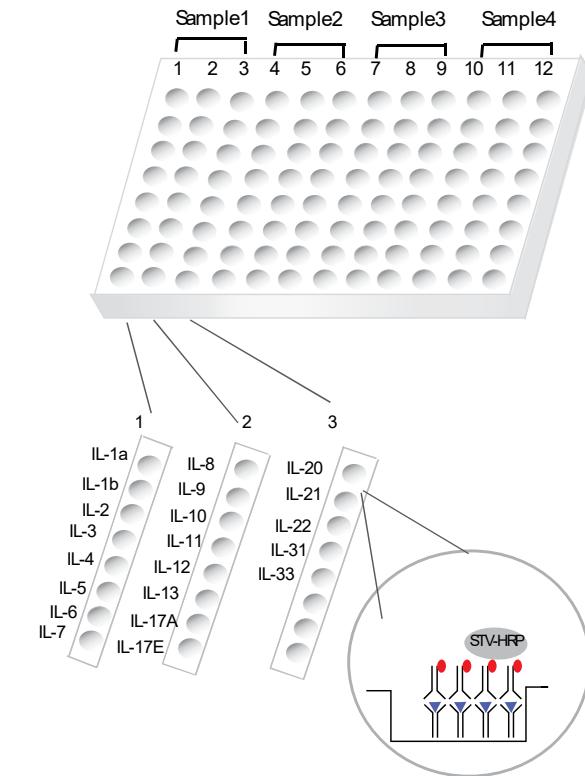


Diagram of Human IL Family ELISA Plate

Materials provided with the kit

Component	Qty	Store at
One clear plate coated with 21 different antibodies against human interleukin cytokines	1	4°C
Biotin labeled antibody mixture against 21 different human interleukin cytokines	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1xDiluent buffer	40 mL	4°C
5X Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

Material required but not provided

- Microplate reader
- Distilled H₂O

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 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 100 µl 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.

Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare enough sample and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200 µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100 µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. 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.**
9. Add 100 µl substrate to each well and incubate for 30-40 minutes at least.

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.

10. Add 50 µl 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.

Diagram of Human Interleukin ELISA Plate Array

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL-1 α	IL-8	IL-20									
B	IL-1 β	IL-9	IL-21									
C	IL-2	IL-10	IL-22									
D	IL-3	IL-11	IL-31									
E	IL-4	IL-12	IL-33									
F	IL-5	IL-13	Blank									
G	IL-6	IL-17a	Blank									
H	IL-7	IL-17E	Blank									