Signosis
Innovative Plate Assay Solutions

Human ICAM-1 ELISA

Catalog Number EA-0518 **(For Research Use Only)**

Introduction

ICAM-1 (Intercellular Adhesion Molecule-1) is a transmembrane glycoprotein primary expressed in immune and endothelial cells. This typical low level expression of this protein is greatly increased by cytokine stimulation. The traditional role of ICAM-1 is to stabilize intercellular adhesion; however ICAM-1 can also bind various immune-associated ligands, is the receptor for rhinovirus, and is believed to have a role in proinflammatory signal transduction.

Principle of the assay

The ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human cytokine antibody for immobilization on the microtiter wells and goat anti-human cytokine antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the molecules of the cytokine 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 concentration of the cytokine is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

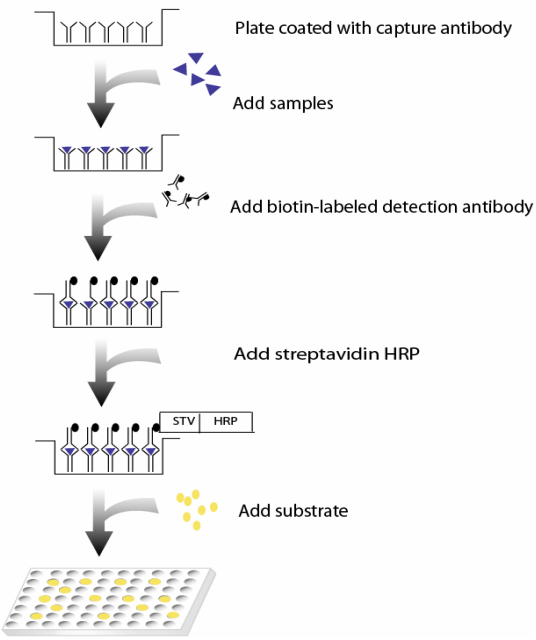


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-well microplate coated with anti-human ICAM-1 antibody	1	4°C
Biotin labeled anti-human ICAM-1 antibody	25µL	-20°C
Recombinant Human ICAM-1 standard (400ng/ml)	10µL	-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	4°C

Material required but not provided

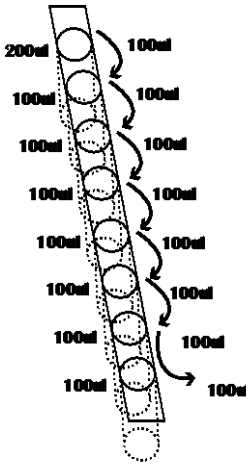
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 100 times of Human recombinant ICAM-1 (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. Add 2ul Human Recombinant ICAM-1 in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute biotin labeled rabbit anti-human ICAM-1 antibodies 1:400 with 1X Diluent buffer before use.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer before use.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Prepare standard according to diagram.



1. Add 200 µl 1X Diluent buffer to the 1st well. Add 100 µl 1x Diluent Buffer to the rest of the wells in the strip.
2. Add 2 µl of standard to the first well.
3. Mix dilution in 1st well and transfer 100 µl from the first well to the 2nd well.
4. Repeat mix and transfer 100 µl into each additional well as pictured.

3. Add 100 µl of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200 µl 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 100 µl of diluted biotin-labeled rabbit anti-human ICAM-1 antibody 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 100 µl of substrate to each well and incubate 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.