Signosis
Innovative Plate Assay Solutions

Human VCAM-1 ELISA

Catalog Number EA-0519 **(For Research Use Only)**

Introduction

VCAM-1 (Vascular Cell Adhesion Molecule-1) is a transmembrane glycoprotein, which is expressed in a variety of cells including fibroblasts, macrophages, endothelial cells, smooth muscle cells, and neurons. In endothelial cells, VCAM-1 is only expressed after cytokine stimulation. The primary roles of VCAM-1 include mediation of leukocyte-endothelial cell adhesion and signal transduction. VCAM-1 has been implicated in atherosclerosis and rheumatoid arthritis.

Principle of the assay

VCAM-1 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-human VCAM-1 for immobilization on the microtiter wells, and biotinated rabbit anti-human VCAM-1 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 VCAM-1 molecules 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 terminated with the addition of Stop Solution changing the color to yellow. The concentration of VCAM-1 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

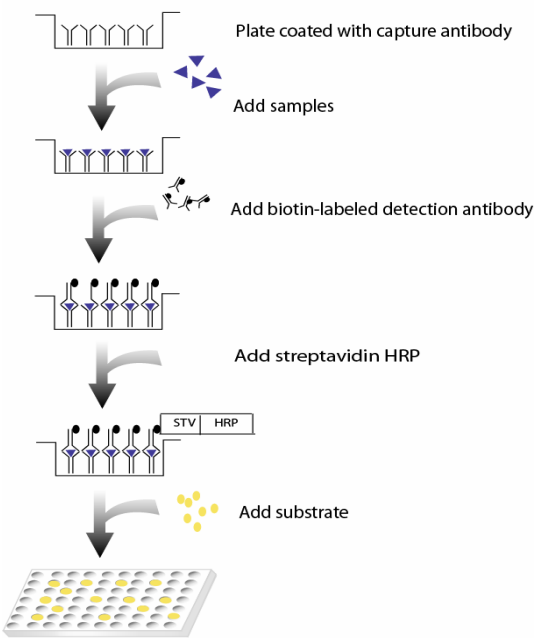


Diagram of ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with rabbit anti-human VCAM-1 antibodies (4ºC)
- Biotin labeled rabbit anti-human VCAM-1 antibodies (-20ºC)
- Streptavidin-HRP conjugate (4ºC)
- Recombinant human VCAM-1 standard (-20ºC)
- 1X Diluent buffer (4ºC)
- 5X Assay wash buffer (4ºC)
- Substrate (4ºC)
- Stop Solution (4ºC)

Material required but not provided

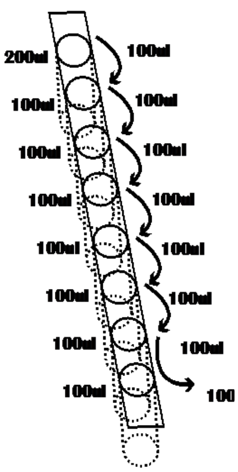
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 50 times of Human recombinant VCAM-1 (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. Add 4ul Human Recombinant VCAM-1 in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute biotin labeled rabbit anti-human VCAM-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 appropriate amount 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 VCAM-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.