

Human VCAM-1/CD106 Sandwich ELISA Kit Datasheet

For the quantitative detection of human VCAM-1/CD106 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00163
Product Name	Human VCAM-1/CD106 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	7412
SwissProt	P19320

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 16000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated(100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4 - 30 mL/bottle. For serum and plasma samples	2 bottles	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

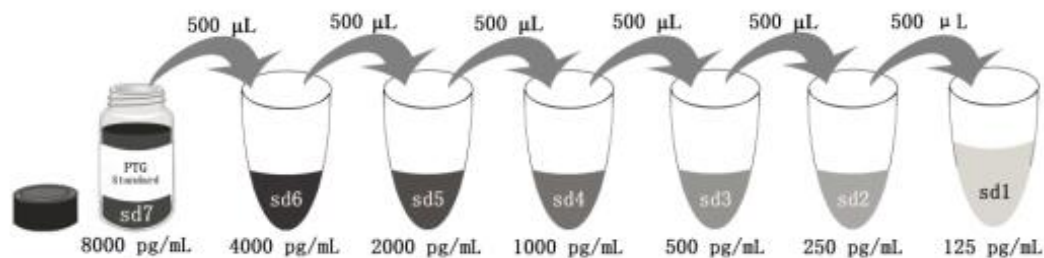
NB: Do not use the kit after the expiration date.

Sample Diluent PT 4 is for protein standard, serum and plasma samples.

Sample Diluent PT 1-ef is for protein standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 4 or PT 1-ef in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4 or PT 1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00163 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The VCAM-1 ELISA kit is to be used to detect and quantify protein levels of endogenous VCAM-1. The assay recognizes human VCAM-1. An antibody specific for VCAM-1 has been pre-coated onto the microwells. The VCAM-1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for VCAM-1 is added to detect the captured VCAM-1 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Vascular cell adhesion molecule 1 (VCAM-1), also known as CD106, is a transmembrane glycoprotein belonging to the immunoglobulin gene superfamily. VCAM-1 is expressed by cytokine-activated endothelium, interacts with integrin VLA4 ($\alpha 4 \beta 1$) present on the surface of leukocytes, and mediates both adhesion and signal transduction. It is also expressed either constitutively or inducibly in a variety of other cell types, including vascular smooth muscle cells, differentiating skeletal muscle cells, renal and neural epithelial cells, macrophages (Kupffer cells), dendritic cells, and bone marrow stromal cells. Soluble VCAM-1 (sVCAM-1) has been found in addition to membrane-bound VCAM-1. Increased sVCAM-1 has been shown to correlate with disease activity in various entities including rheumatoid arthritis, systemic lupus erythematosus, and lupus nephritis.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:100 or 1:200 dilution is recommended for serum or

plasma. 1:2 dilution is recommended for cell culture supernatants.

Safety Notes

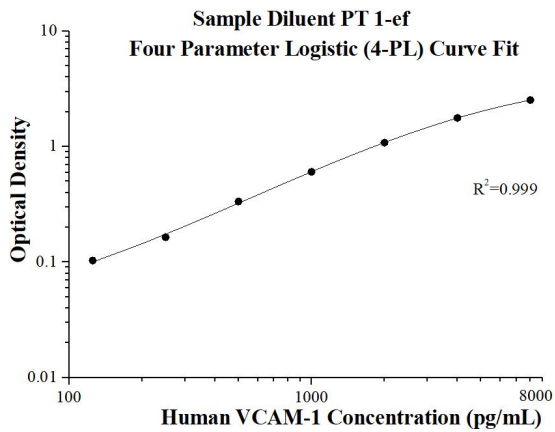
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

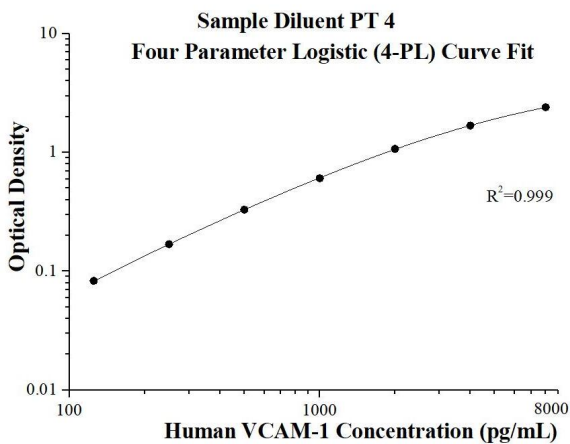
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.079 0.077	0.078	-
125	0.185 0.176	0.181	0.103
250	0.267 0.216	0.242	0.164
500	0.455 0.370	0.413	0.335
1000	0.678 0.685	0.682	0.604
2000	1.169 1.153	1.161	1.083
4000	1.923 1.779	1.851	1.773
8000	2.613 2.610	2.612	2.534



(pg/mL)	O.D	Average	Corrected
0	0.069 0.068	0.069	-
125	0.153 0.150	0.152	0.083
250	0.242 0.232	0.237	0.169
500	0.397 0.399	0.398	0.330
1000	0.673 0.678	0.676	0.607
2000	1.174 1.105	1.140	1.071
4000	1.765 1.743	1.754	1.686
8000	2.510 2.430	2.470	2.402

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	2,573.5	66.7	2.6	1	24	2,472.2	69.7	2.8
2	20	893.2	19.3	2.2	2	24	772.0	42.8	5.5
3	20	209.0	8.8	4.2	3	24	193.4	9.9	5.1

Recovery

The recovery of VCAM-1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:160	101	96-106
	1:320	99	82-113
Cell culture supernatants	1:2	84	75-92
	1:4	86	75-105

Sample Values

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=24)	235	106-532

Cell Culture Supernates - Human peripheral blood cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human VCAM-1. No detectable levels were observed.

Sensitivity

The minimum detectable dose of human VCAM-1 is 1.7 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, human plasma samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants were spiked with high concentrations of VCAM-1 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The human plasma samples were initially diluted 1:20)

		Human plasma (Sample Diluent PT 4)	Cell culture supernatants (Sample Diluent PT1-ef)
1:2	Average% of Expected	100	82
	Range (%)	-	75-90
1:4	Average% of Expected	106	88
	Range (%)	99-111	86-90
1:8	Average% of Expected	103	87
	Range (%)	99-108	80-95
1:16	Average% of Expected	104	99
	Range (%)	100-107	96-101

References

1. Elices MJ, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell*. 60(4):577-84 (1990).
2. Pigott R, et al. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun*. 187(2):584-9 (1992).
3. Wellicome SM, et al. Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus. *Clin Exp Immunol*. 92(3):412-8 (1993).
4. Cybulsky MI, et al. Structure of the murine VCAM1 gene. *Genomics*. 18(2):387-91 (1993).
5. Spronk PE, et al. Levels of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin during disease exacerbations in patients with systemic lupus erythematosus (SLE); a long term prospective study. *Clin Exp Immunol*. 97(3):439-44 (1994).
6. Ikeda Y, et al. Relationship between lupus nephritis activity and the serum level of soluble VCAM-1. *Lupus*. 7(5):347-54 (1998).
7. Tu Z, et al. I kappa B kinase is critical for TNF-alpha-induced VCAM1 gene expression in renal tubular epithelial cells. *J Immunol*. 166(11):6839-46 (2001).