Mouse VCAM-1/CD106 Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE10038
Product Name	Mouse VCAM-1/CD106 Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	22329
SwissProt	P29533

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 - 1000 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	2 bottles	
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	

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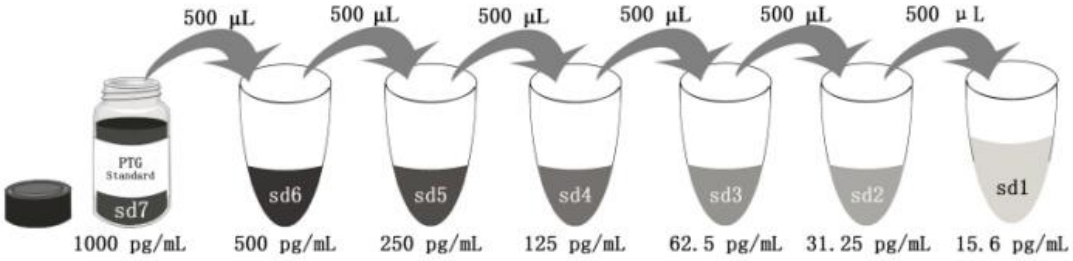
Sample Diluent PT 4 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 4 in standard. This reconstitution gives a stock solution of 1000 pg/mL.



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	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE10038 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 mouse 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 of biotinylated 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. The VCAM-1/integrin VLA4 ($\alpha 4\beta 1$) interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation. VCAM-1 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.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2,000 dilution is recommended for serum or plasma.

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1:2 dilution is recommended for cell culture supernatants .

Safety Notes

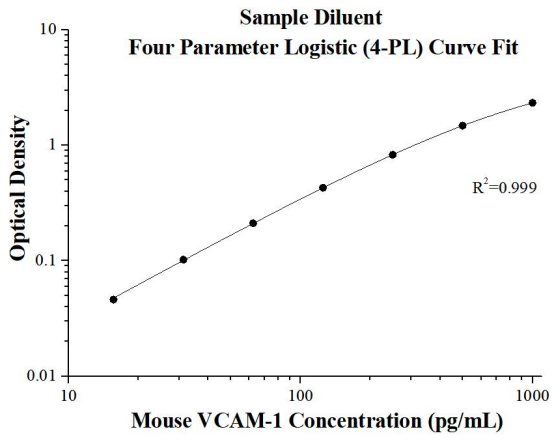
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.021 0.019	0.020	-
15.6	0.066 0.065	0.066	0.046
31.25	0.124 0.120	0.122	0.102
62.5	0.234 0.227	0.231	0.211
125	0.453 0.445	0.449	0.429
250	0.846 0.849	0.848	0.828
500	1.503 1.496	1.500	1.480
1000	2.367 2.332	2.350	2.330

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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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	766.0	23.0	3.0
2	20	412.4	6.3	1.5
3	20	317.8	6.5	2.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	689.0	48.9	7.1
2	24	375.9	28.4	7.6
3	24	283.9	20.0	7.0

Recovery

The recovery of VCAM-1 spiked to three different levels in four samples throughout the range of the assay in plasma and cell culture supernatants was evaluated. (Serum was diluted 1:1000 prior to assay).

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:2	102	96-118
	1:4	101	96-109
Cell culture supernatants	1:2	92	88-98
	1:4	92	85-100

Sample Values

Sample Type	Mean of Detectable (**ng/mL**)	Range (**ng/mL**)
Mouse serum (n=30)	398.5	326.3-555.5

Cell Culture Supernates:L-929 mouse fibroblast cells (1 x 10^6 cells/mL) were cultured for 3 days in MEM supplemented with 10% equine serum . An aliquot of the cell culture supernate was removed, assayed for mouse VCAM-1, and measured 193 pg/mL.

Sensitivity

The minimum detectable dose of mouse VCAM-1 is 1.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, Mouse serum samples and cell culture supernatants were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The mouse serum samples were initially diluted 1:1000)

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	107	113
	Range (%)	106-109	110-115
1:8	Average% of Expected	113	118
	Range (%)	110-116	117-119
1:16	Average% of Expected	116	111
	Range (%)	113-119	97-112

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. Cybulsky MI, et al. Structure of the murine VCAM1 gene. Genomics. 18(2):387-91 (1993).
4. 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).

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