Validation Report #029702

Validation Date: 05/17/14

Summary

Antigen	Vascular Cell Adhesion Molecule 1 (VCAM1)
Catalog number	ABIN367720
Supplier	Cusabio
Supplier catalog number	csb-e04754m
Lot number	W22184081
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Celplor LLC
Validation number	029702
Positive Control	Normal mouse serum
Negative Control	Normal horse serum (non-reactive species)
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody

- Antigen: Vascular Cell Adhesion Molecule 1 (VCAM1)
- Catalog number: ABIN367720
- Supplier: Cusabio
- Supplier catalog number: csb-e04754m
- Lot number: W22184081

Controls

- Positive control: Mouse serum (Jackson ImmunoResearch, Cat# 015-000-001, Lot# 112574)
- Negative control: Horse serum (In house stock from healthy horse)
- Spike Control: Standard spiked into sample diluent buffer.

Protocol

- All reagents in the ELISA kit were brought up to room temperature (RT) before use.
- 100 µL of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in triplicate. Plate was covered with sealer (provided in kit) and incubated for 2h at 37 °C.
- After incubation, liquid in each well was removed by suction.
- 100 µL of Biotin-antibody (1X) was added per well. Plate was covered with sealer and incubated for 60 min at 37 °C.
- Wells were washed with 200 µL of wash buffer three times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 100 µL of HRP-avidin (1x) was added per well. The plate was covered with sealer and incubated for 60 mins at 37°C.
- Wells were washed with 200 µL of wash buffer five times same as step 5.
- 90 µL of TMB Substrate was added to each well and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated at 37°C in the dark.
- After 30 mins, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µL of Stop Solution to each well.
- The optical density (OD value) of each well was immediately read using a micro-plate reader set to 450 nm (with reference set to 570 nm).
- The triplicate readings for each standard were averaged and the average zero standard optical density subtracted. A standard curve was generated by plotting the normalized OD value for each standard on the y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation x = (y-0.0197) / 0.0002. The equation x = (y-0.0197) / 0.0002 was used to calculate VCAM-1 concentrations of the samples based on their normalized average OD values.

Experimental Notes

- No challenges noted.

Figures

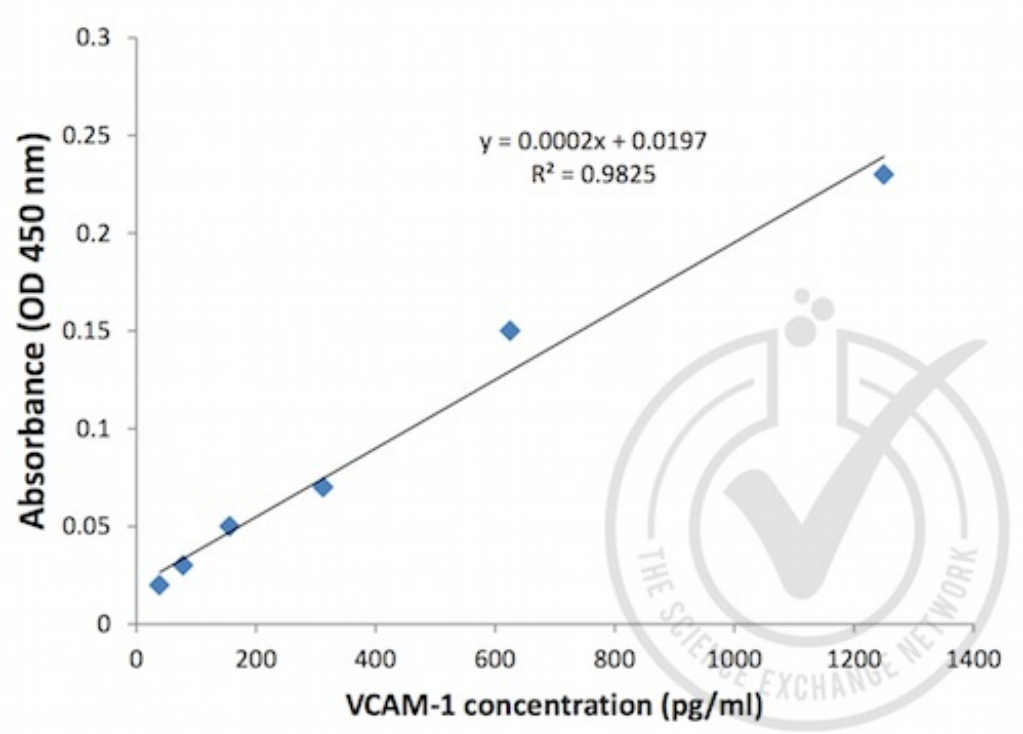

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings (excluding readings from 0 and 2500 pg/ml) plotted for standard curve samples. Linear range is between 39.0062 and 1250 pg/ml.

Type	sample	reading1	reading2	reading3	SD	Average	Normalized Average	Calculated Conc (pg/ml)
standard curve	2500 pg/ml	0.431	0.412	0.413	0.010693	0.42	0.37	1744.83
	1250 pg/ml	0.299	0.252	0.288	0.024583	0.28	0.23	1049.83
	625 pg/ml	0.173	0.197	0.214	0.020599	0.19	0.14	624.83
	312.5 pg/ml	0.099	0.134	0.141	0.022502	0.12	0.07	274.83
	156.25 pg/ml	0.097	0.088	0.11	0.01106	0.10	0.05	143.17
	78.125 pg/ml	0.089	0.078	0.072	0.008622	0.08	0.03	49.83
	39.062 pg/ml	0.062	0.08	0.074	0.009165	0.07	0.02	11.50
	0 ng/ml	0.047	0.049	0.054	0.003606	0.05	0.00	-98.50
spike control	625 ng/ml	0.177	0.194	0.172	0.011533	0.18	0.12	506.50
Sample diluent	0 ng/ml	0.064	0.061	0.061	0.001732	0.06	0.00	-88.50
positive control	MS (1:100)	0.708	0.69	0.863	0.095112	0.75	0.69	3369.83
	MS (1:200)	0.396	0.448	0.47	0.038	0.44	0.38	1791.50
	MS (1:500)	0.219	0.223	0.208	0.007767	0.22	0.16	684.83
negative control	HS (1:100)	0.055	0.047	0.051	0.004	0.05	-0.01	-143.50
	HS (1:200)	0.061	0.054	0.055	0.003786	0.06	0.00	-115.17
	HS (1:500)	0.062	0.058	0.056	0.003055	0.06	0.00	-105.17

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown positive (normal mouse serum) and negative (normal horse serum) control samples. Value for Average Reading is derived from the average of three readings (OD 450nm). The Average Reading for 0ng/ml Standard was subtracted from all Average Readings of other Standards to yield normalized Average Absorbance values for Standards. The Average Reading for Sample Dilution buffer was subtracted from Average Readings of spike control, mouse serum and horse serum to yield normalized Average Absorbance values for these samples. Standard deviation is included for all samples. An equation X=(Y-0.0197)/0.0002 was generated from the standard curve and used to calculate VCAM-1 concentrations shown in the Table. Undiluted VCAM-1 concentration in normal mouse serum was calculated from data obtained in 1:500 diluted mouse serum.