Validation Report #029793

Validation Date: 08/12/14

Summary

Antigen	Vascular Cell Adhesion Molecule 1 (VCAM1)
Catalog number	ABIN366645
Supplier	Cusabio
Supplier catalog number	csb-e04753h
Lot number	Z02184068
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029793
Positive Control	Human serum - expression is ~280 ng/mL
Negative Control	Goat serum (non-reactive species)
Notes	Target protein was detected in the positive control sample and not in the negative control sample as expected.

Full Methods

ELISA kit

- Antigen: Vascular Cell Adhesion Molecule 1 (VCAM1)
- Catalog number: ABIN366645
- Supplier: Cusabio
- Supplier catalog number: csb-e04753h
- Lot number: Z02184068

Controls

- Positive control: Human serum (Sigma Aldrich, Cat# H6914-20ML, Lot# SLBK2170V)
- Negative control: Goat serum (Sigma Aldrich, Cat# G9023-10ML, Lot# SLBH2670V)

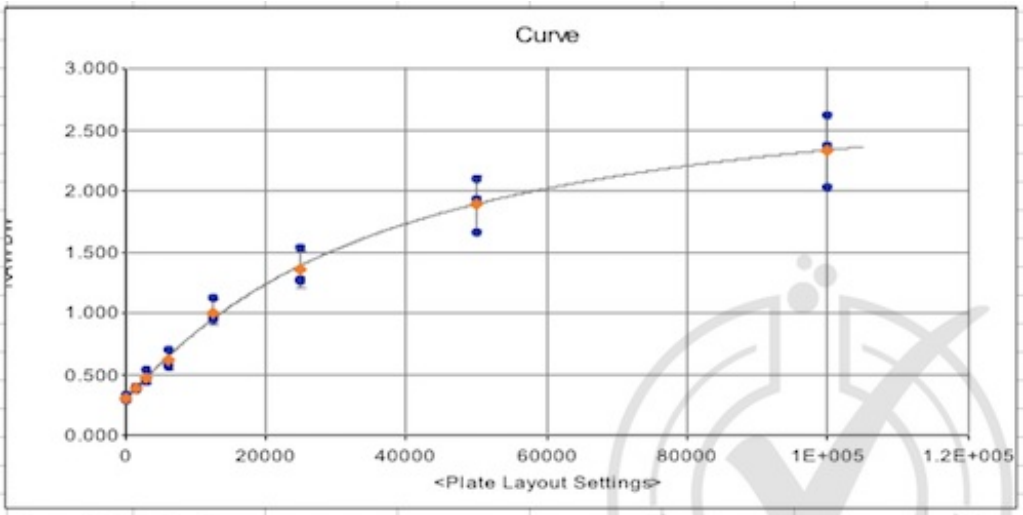
Protocol

1. All reagents were brought up to room temperature for 30 minutes prior to use. The 1x Wash Buffer was prepared by adding 20 mL of 25x Wash Buffer Concentrate to 480 mL of distilled/deionized water and mixing thoroughly.
2. The vial of Standard was reconstituted with 1 mL of Sample Diluent, mixed, and allowed to sit for 15 minutes with gentle agitation.
3. The standard curve was prepared by creating a 2-fold dilution series of seven standards (including the original undiluted vial) using Sample Diluent. Sample Diluent alone served as the 0 pg/mL standard.
4. The assay plate was removed from the foil pouch and 100 µL of each standard and sample were added to the appropriate wells, in triplicate. The plate was covered with the adhesive strip provided and incubated for 2 hours at 37 °C.
5. Approximately 10 minutes before the incubation ended, a 1x Biotin-antibody solution was prepared by diluting 60 µL of 100x Biotin-antibody into 5940 µL of Biotin-antibody Diluent.
6. The liquid from each well was removed.
7. 100 µL of 1x Biotin-antibody solution was added to each well, and the plate was covered with a new adhesive strip, and incubated for 1 hour at 37 °C.
8. Approximately 10 minutes before the incubation ended, a 1x HRP-avidin solution was prepared by diluting 60 µL of 100x HRP-avidin into 5940 µL of HRP-avidin Diluent.
9. Each well was aspirated and washed, repeating the process two times for a total of three washes. Each well was washed by filling each well with 1x Wash Buffer and letting it stand for 2 minutes. After the last wash, remaining Wash Buffer was removed and the plate was inverted and blotted against clean, absorbent paper towels.
10. 100 µL of 1x HRP-avidin solution was added to each well, the plate was covered with a new adhesive strip, and incubated for 1 hour at 37 °C.
11. The aspiration/wash procedure from Step 9 was repeated for an additional 5 washes.
12. 90 µL of TMB Substrate was added to each well. The plate was protected from light and incubated for 15-30 minutes at 37 °C, with periodic checking to prevent overdevelopment.
13. 50 µL of Stop Solution was added to each well and mixed thoroughly. The optical density (OD) of each well was measured within 5 minutes using a microplate reader set to 450 nm.
14. A standard curve was generated by plotting the OD value for each standard on the y-axis against the concentration on the x-axis. A line of best fit through the points on the graph was used to generate an equation to calculate VCAM1 concentrations of the samples based on their average OD values.

Experimental Notes

No experimental challenges noted.

Figures



Curve Fitting Results

Curve Name	Curve Formula	A	B	C	D	R2
Curve	Y = (A-D)/(1+(X/C)^B) + D	0.301	1.09	3.58E+04	3.01	0.999

Figure 1: VCAM1 standard curve graph and equation.

Layout

	1	2	3	4	5	6	
A	STD1	STD1	STD1	SPL1:1	SPL1:1	SPL1:1	Well ID
	0	0	0	1	1	1	Conc/Dil
				Human serum	Human serum	Human serum	Name
B	STD2	STD2	STD2	SPL1:2	SPL1:2	SPL1:2	Well ID
	1562.5	1562.5	1562.5	2	2	2	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	3125	3125	3125	5	5	5	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	6250	6250	6250	10	10	10	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5	STD5	STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	12500	12500	12500	20	20	20	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	25000	25000	25000	40	40	40	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	50000	50000	50000	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	1.00E+05	1.00E+05	1.00E+05	1	1	1	Conc/Dil
				Goat serum	Goat serum	Goat serum	Name

Figure 2: Plate layout. Standard concentrations are in pg/mL; serum dilution values indicate their fold change from the undiluted stock.

RAW DW

	1	2	3	4	5	6	
A	0.288	0.296	0.341	1.433	1.794	1.754	RAW DW
B	0.375	0.396	0.4	1.689	1.835	1.974	RAW DW
C	0.444	0.451	0.537	1.455	1.361	1.715	RAW DW
D	0.582	0.564	0.711	1.32	1.389	1.418	RAW DW
E	0.966	0.947	1.125	0.917	0.981	1.007	RAW DW
F	1.283	1.269	1.543	0.581	0.637	0.612	RAW DW
G	1.935	1.661	2.109	0.491	0.563	0.524	RAW DW
H	2.371	2.028	2.626	0.058	0.055	0.056	RAW DW

Figure 3: Raw OD readings of standards and controls.

Conc

	1	2	3	4	5	6	
A	<0.000	<0.000	739.706	26381.86	43277.71	40966.16	Conc
B	1324.679	1681.668	1749.537	37487.66	45798.57	55721.62	Conc
C	2496.713	2616.004	4102.746	27204.41	23829.29	38840.25	Conc
D	4902.081	4580.262	7305.024	22463.63	24797.6	25832.87	Conc
E	12717.82	12277.17	16699.14	11595.05	13070.59	13692.62	Conc
F	21281.23	20845.55	30718.33	4884.125	5904.571	5445.152	Conc
G	52695.49	36085.02	68083.47	3301.925	4562.467	3874.903	Conc
H	>105000.000	60282.68	>105000.000	<0.000	<0.000	<0.000	Conc

Conc x Dil

	1	2	3	4	5	6	
A				26381.86	43277.71	40966.16	Conc x Dil
B				74975.33	91597.13	111443.2	Conc x Dil
C				136022	119146.5	194201.2	Conc x Dil
D				224636.3	247976	258328.7	Conc x Dil
E				231901	261411.7	273852.3	Conc x Dil
F				195365	236182.8	217806.1	Conc x Dil
G				165096.2	228123.3	193745.2	Conc x Dil
H				<0.000	<0.000	<0.000	Conc x Dil

Figure 4: VCAM1 concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 170116 pg/mL (170 ng/mL) of VCAM1 was detected in the positive control (human serum) and 0 pg/mL of VCAM1 was detected in the negative control (goat serum).