Validation Report #029475

Validation Date: 12/03/13

Summary

Antigen	Angiopoietin-like 2 (ANGPTL2)
Catalog number	ABIN578045
Supplier	Cusabio
Supplier catalog number	csb-e13881h
Lot number	T30092379
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories Inc
Validation number	29475
Positive Control	Human sera
Negative Control	Goat Sera
Notes	Signal was detected in positive control samples but not in negative control samples.

Full Methods

Primary Antibody

- Antigen: Angiopoietin-Like 2 (ANGPTL2)
- Catalog Number: ABIN578045
- Supplier: Cusabio
- Supplier Number: csb-e13881h
- Lot Number: T30092379

Controls

- Positive control: Serum from normal adult human (specimen known to contain the target protein).
- Negative control: Serum from normal goat (specimen known to not contain the target protein).
- Standard curve: Serial two-fold dilutions from 100 ng/ml [100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0] were generated from the standard provided in the kit using standard/sample diluent buffer.
- Spike control: Standard diluted in standard/PBS diluent buffer [50 and 0].

Protocol

- All reagents in the ELISA kit were brought up to room temperature (RT) before use.
- 100 µL of standard or sample were added to wells in ELISA plate pre-coated with capture antibody. All samples and standards were assayed in triplicate.
- The plate was covered with sealer (provided in kit) and incubated for 2 h at 37°C. Unbound material was aspirated and 100 µL of Biotin-Antibody (diluted 1:100 in "Diluent for Biotinylated Detection Ab") was added to each well. Plate was sealed and incubated for 1 h at 37°C. Unbound Biotin-Antibody was removed from each well and plate was washed three times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 100 µL of HRP-Conjugate (1X) was added to each well. Plate was sealed and incubated for 1 h at 37°C.
- After 1 h incubation at 37°C, unbound HRP-Avidin was removed by washing five times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 90 µL of TMB substrate was added to wells and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated for 25 min at 37°C in the dark.
- After 25 min, 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 read using a microplate reader set to 450 nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted to yield 'corrected absorbance at 450 nm'. A standard curve was generated by plotting the mean OD value for each standard on the X-axis against the concentration on the Y-axis using Excel. Standard curve was generated by regression analysis with four-parameter logistic.
- An equation ($y = 11.973x4 - 32.453x3 + 42.158x2 + 32.504x + 0.073$) was derived from the standard curve and used to calculate Angiopoietin-Like 2 (ANGPTL2) concentrations in samples based on their Average Absorbance values.

Experimental Notes

Nothing to note.

Figures

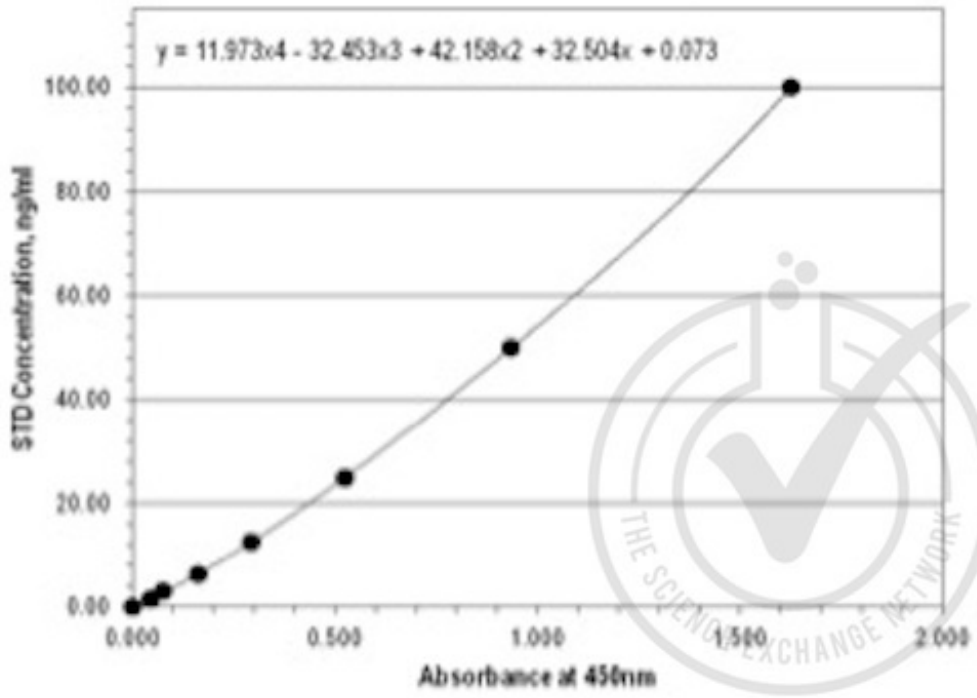

Figure 1: Graph of Corrected OD450 nm plotted for standard curve samples.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD_{450nm}	SD	Calculated conc ng/ml
		1	2	3				
Standard Curve	100.00	1.648	1.668	1.562	1.626	1.626	0.056	108.56
	50.00	0.952	1.101	0.983	1.012	0.936	0.079	50.01
	25.00	0.583	0.601	0.618	0.601	0.525	0.018	24.95
	12.50	0.321	0.415	0.375	0.370	0.294	0.047	12.55
	6.25	0.269	0.216	0.234	0.240	0.164	0.027	6.39
	3.13	0.132	0.139	0.186	0.152	0.076	0.029	2.79
	1.56	0.120	0.145	0.102	0.122	0.046	0.022	1.67
	0.00	0.074	0.078	0.077	0.076	0.000	0.002	0.08
Spike Controls	50.00	1.025	1.118	0.935	1.026	0.950	0.092	50.93
	0.00	0.058	0.055	0.053	0.055	-0.021	0.003	-0.58
Samples	PBS	0.069	0.101	0.069	0.080	0.004	0.018	0.19
	Serum, Human	0.484	0.453	0.468	0.468	0.392	0.016	17.64
	Serum, Goat	0.066	0.065	0.068	0.066	-0.010	0.002	-0.24

Conc of ANGPTL2 in Human Serum (+ ve Control) : 17.64 ng/ml
Conc of ANGPTL2 in Goat Serum (-ve Control) : -0.24 ng/ml

Table 1: ELISA. ANGPTL2 is present in Human serum and undetectable in goat serum. Spike controls indicate no interference in absorbance readings from the diluent used to prepare standards and sera samples. Absorbance readings (OD450 nm) are shown for standard curve, spike controls and unknown samples. Value for avg reading is derived from the average reading of three samples. Avg reading for 0 ng/ml Standard was subtracted from all avg readings to yield “Corrected OD450 nm “values for Standards, spike controls and unknown samples. Standard

deviation is included for all samples. Standard curve was generated by regression analysis with four-parameter logistic. An equation (y = 11.973x4 - 32.453x3 + 42.158x2 + 32.504x + 0.073) was derived from the standard curve and used to calculate ANGPTL2 concentrations shown in the Table 1.