Validation Report #028773

Validation Date: 10/02/13

Summary

Antigen	Interleukin 6 (IL-6)
Catalog number	ABIN365163
Supplier	Cusabio
Supplier catalog number	csb-e04638h
Lot number	Q03097538
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories Inc
Validation number	28773
Positive Control	Human serum
Negative Control	Mouse brain lysate
Notes	Signal was detected in positive control samples but not in negative control samples.

Full Methods

Primary Antibody

- Antigen: Interleukin 6 (IL-6)
- Catalog number: ABIN365163
- Supplier: Cusabio
- Supplier catalog number: csb-e04638h
- Batch number: Q03097538

Controls

- Positive control: human serum (specimen known to contain the target protein) was prepared by Alamo Laboratories.
- Negative control: protein extract from mouse brain (specimen known to not contain the target protein) was prepared by Alamo Laboratories.
- Standard curve: serial two-fold dilutions from 500 pg/ml [500, 250, 125, 62.5, 31.25, 15.6, 7.8, 0] were generated from the standard provided in the kit using sample diluent buffer.
- Spike control: standard diluted in PBS [62.5 and 0 pg/mL].

Protocol

- To each well, 100 µL of standard or sample were added. Plate was incubated for 2 h at 37°C. The liquid from each well was aspirated but wells were not washed.
- 100 µL of Biotin-antibody (1x) was added to each well and the plate was incubated for 1 h at 37°C.
- The liquid from each well was aspirated and wells were washed three times with 200 µL of Wash Buffer (1X) each time x 2 min.
- 100 µL of HRP-avidin (1x) was added to each well and the plate was incubated for 1 h at 37°C.
- The liquid from each well was aspirated and wells were washed five times with 200 µL of Wash Buffer (1X) each time x 2 min.
- 90 µL of TMB Substrate was added to each well and the plate was incubated for 25 min at 37°C.
- 50 µL of Stop Solution to each well and the contents were mixed by tapping gently. Absorbance of each well was measured at 450 nm and 540 nm within 5 min using a microplate reader.
- The readings at 540 nm were subtracted from those at 450 nm to correct for optical imperfections in the plate.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. The corrected average-value was tabulated as Average Absorbance. 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 = 248.75x4-542.17x3+396.6x2+131.48x+2.0519) was derived from the standard curve and used to calculate IL-6 concentrations based on their Average Absorbance values.

Experimental Notes

None

Figures

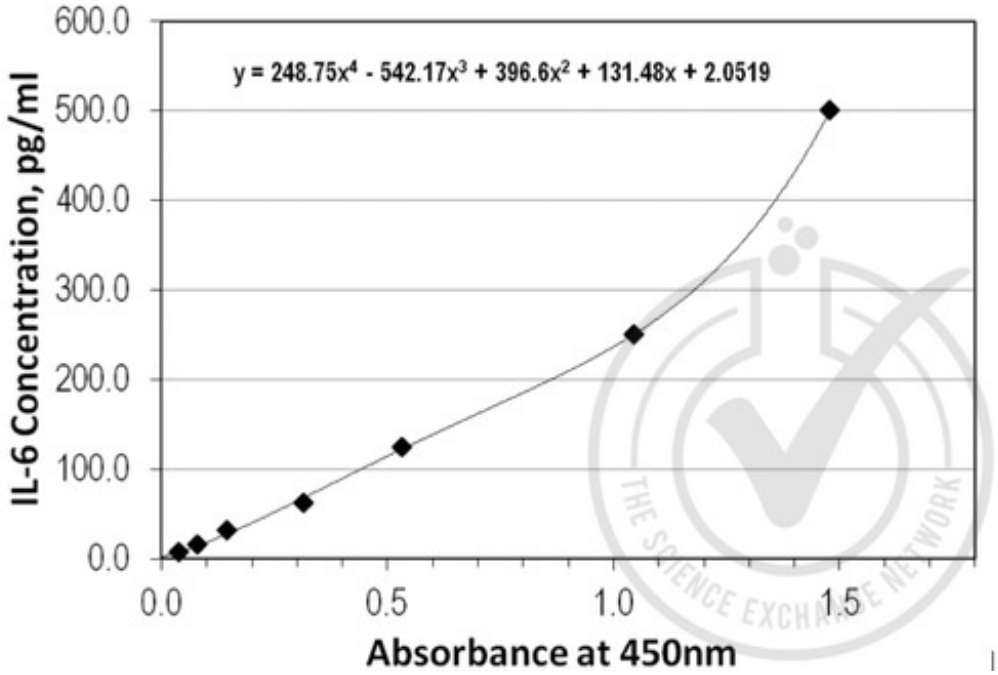

Figure 1: Graph of corrected-average readings (OD 450 nm) plotted for standard curve samples.

Type	Sample pg/ml	Reading-1		Reading-2		Reading-3		Corrected Readings at 450 nm (OD_{450nm} - OD_{540nm})			Avg Reading	Avg Absorbance	SD	Calculated conc of IL-6, pg/ml
		450 nm	540 nm	450 nm	540 nm	450 nm	540 nm	Reading -1	Reading -2	Reading -3				
Standard Curve	500.0	1.567	0.051	1.599	0.048	1.620	0.044	1.516	1.551	1.576	1.548	1.479	0.030	500
	250.0	1.189	0.046	1.196	0.048	1.091	0.041	1.143	1.148	1.050	1.114	1.045	0.055	250
	125.0	0.588	0.046	0.647	0.037	0.700	0.050	0.542	0.610	0.650	0.601	0.532	0.055	122
	62.5	0.389	0.048	0.491	0.041	0.394	0.038	0.341	0.450	0.356	0.382	0.313	0.059	68
	31.2	0.288	0.052	0.217	0.044	0.265	0.039	0.236	0.173	0.226	0.212	0.143	0.034	27
	15.6	0.191	0.036	0.164	0.039	0.200	0.039	0.155	0.125	0.161	0.147	0.078	0.019	14
	7.8	0.162	0.050	0.134	0.039	0.147	0.034	0.112	0.095	0.113	0.107	0.038	0.010	8
	0.0	0.128	0.041	0.101	0.039	0.093	0.036	0.087	0.062	0.057	0.069	0.000	0.016	2
Spike Controls	62.5	0.419	0.041	0.427	0.043	0.391	0.042	0.378	0.384	0.349	0.370	0.301	0.019	65
	0.0	0.151	0.039	0.106	0.040	0.102	0.042	0.112	0.066	0.060	0.079	0.010	0.028	3
Samples	PBS	0.102	0.039	0.120	0.042	0.122	0.039	0.063	0.078	0.083	0.075	0.006	0.010	3
	Serum Hs	0.164	0.036	0.175	0.041	0.156	0.035	0.128	0.134	0.121	0.128	0.059	0.007	11
	Brain, Mm	0.118	0.036	0.109	0.038	0.112	0.037	0.082	0.071	0.075	0.076	0.007	0.006	3

Table 1: ELISA. IL-6 is present in the positive control sample (human serum) and absent from the negative control sample (mouse brain). Spike controls indicate no interference in absorbance readings from the protein lysate buffer used to prepare the protein extracts. Absorbance readings (OD 450 nm) are shown for standard curve, spike controls and unknown positive and negative control samples. The absorbance of all the samples including standards, spike controls and unknown samples were measured at 450 and 540 nm and the absorbance values at 540 nm were subtracted from those at 450 nm to account for optical imperfections in the ELISA plate. Value for Average Reading is derived from the average of three corrected-readings (OD 450nm). The Average Reading for 0 ng/ml Standard was subtracted from all Average Readings to yield Average Absorbance 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 = 248.75x4 - 542.17x3 + 396.6x2 + 131.48x + 2.0519) was derived from the standard curve and used to calculate IL-6 concentrations shown in the Table.