Validation Report #029604

Validation Date: 02/08/14

Summary

Antigen	Human Lipocalin 2 (LCN2)
Catalog number	ABIN365998
Supplier	Cusabio
Supplier catalog number	CSB-E09408h
Lot number	U26095947
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories Inc
Validation number	29604
Positive Control	Human serum
Negative Control	Goat serum
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody

- Antigen: human Lipocalin 2 (LCN2)
- Catalog number: ABIN365998
- Supplier: Cusabio
- Supplier catalog number: CSB-E09408h
- Lot number: U26095947

Controls

- Positive control: Serum from normal adult human (specimen known to contain the target protein).
- Negative control: Serum from normal goat (specimens known to not contain the target protein).
- Standard curve: Serial two-fold dilutions from 2000 pg/ml [2000, 1000, 500, 250, 125, 62.5, 31.25, 0] were generated from the standard provided in the kit using standard/sample diluent buffer.
- Spike control: Standard diluted in standard/PBS diluent buffer [250 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 hours at 37°C. Unbound material was aspirated but the wells were NOT Washed.
- 100 µL of Biotin-Antibody (diluted 1:100 in “Biotin-Antibody Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour 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-Avidin Conjugate (diluted 1:100 in “HRP-Avidin Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour 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 and blotted 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 gently tapped to ensure mixing and incubated for 30 min at 37°C in the dark.
- After 20 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 = 217.78x4 - 398.83x3 + 327.6x2 + 594.62x) was derived from the standard curve and used to calculate LCN2 concentrations in samples based on their Average Absorbance values.

Experimental Notes

None

Figures

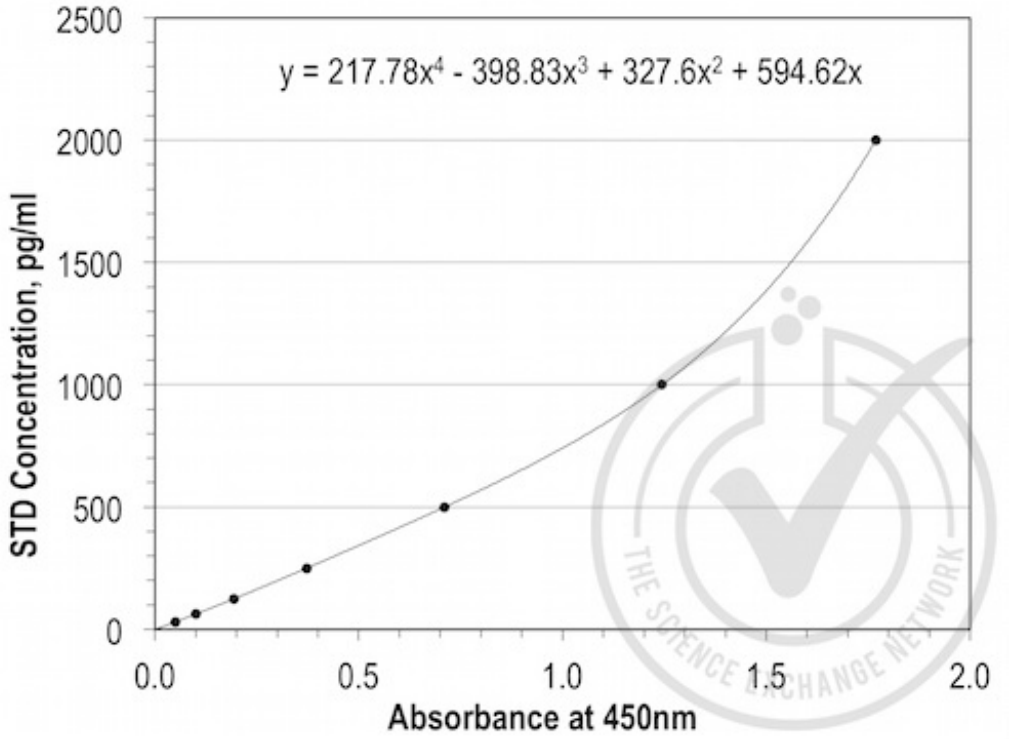

Figure 1: Graph of corrected OD 450 nm plotted for standard curve samples.

Type	Sample, pg/ml	Readings at 450 nm			Avg Reading	Corrected OD_{450nm}	SD	Calculated conc pg/ml
		1	2	3				
Standards	2000	1.875	1.878	1.801	1.851	1.768	0.036	2000.01
	1000	1.295	1.334	1.351	1.327	1.244	0.023	1000.02
	500	0.797	0.779	0.803	0.793	0.710	0.010	499.92
	250	0.454	0.448	0.463	0.455	0.372	0.006	250.17
	125	0.278	0.269	0.283	0.277	0.194	0.006	124.85
	62.5	0.188	0.185	0.178	0.184	0.101	0.004	62.79
	31.25	0.134	0.135	0.131	0.133	0.050	0.002	30.71
	0	0.082	0.081	0.085	0.083	0.000	0.002	-0.20
Spike Controls	250.00	0.466	0.433	0.430	0.443	0.360	0.016	241.57
	0.00	0.088	0.089	0.091	0.089	0.006	0.001	3.78
Test Samples	Serum, Human	4.015	4.200	2.894	3.703	3.620	0.577	24924.19
	Serum, Human, 1:500 Diluted	0.510	0.511	0.516	0.512	0.429	0.003	291.51
	Serum, Goat	0.081	0.083	0.081	0.082	-0.001	0.001	-0.79

NGAL conc in Human Serum, 1:500 dilu (+ve Control): 291.51pg x 500 (Dilu factor)/1000= 146 ng/ml
*NGAL conc in Human Serum, undiluted (+ve Control) : 24,924 pg/ml **Out of STD Conc range!***
NGAL conc in goat Serum (-ve Control) : -0.79 pg/ml = - 0.00079 ng/ml

Table 1: ELISA. Lipocalin 2 (LCN2) 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 (OD 450 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” amount of 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 = 217.78x4 - 398.83x3 + 327.6x2 + 594.62x) was derived from the standard curve and used to calculate Lipocalin 2 (LCN2) concentrations shown in Table 1.