Validation Report #029583

Validation Date: 01/30/14

Summary

Antigen	NGAL/MMP-9 complex
Catalog number	ABIN1690717
Supplier	Cusabio
Supplier catalog number	CSB-EQ01026975HU
Lot number	T30095914
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies
Validation number	29583
Positive Control	Human individual postmenopausal female serum
Negative Control	Chicken plasma
Notes	Standard curve was fit to a logistic equation, which returned a small positive concentration value for the negative control.

Full Methods

Primary Antibody

- Antigen: NGAL/MMP-9 complex
- Catalog number: ABIN1690717
- Supplier: Cusabio
- Supplier catalog number: CSB-E08006h
- Lot number: T23095910

Controls

- Positive control: Human individual post-menopausal female serum (Biochemed, 750-NS-FI-POM)
- Negative control: Chicken plasma (Sigma-Aldrich, p3266) – reconstituted at 1 mg/mL
- Standard curve: 0, 312, 0.625, 1.25, 2.5, 5, 10, 20 ng/mL NGAL/MP9 complex provided in the ELISA kit
- Spike control: 10 ng/mL standard premixed with chicken plasma in a 1:1 ratio

Protocol

- 100 µL of standard and samples were added 96-well strip plates provided in the kit. All samples and standards were assayed in duplicate.
- The microplate was covered and incubated at 37°C for 1 hr.
- Plate contents were discarded.
- 100 µL of biotin antibody conjugate was added and incubated at 37°C for 1 hr.
- Content of the wells was discarded and wells were washed 3 times with 200 µl of 1x wash solution with a 2 min soak for each wash.
- 100 µL of HRP-avidin conjugate was added and incubated at 37°C for 1 hr.
- Contents of the wells were discarded and wells were washed 5 times with 200 µl of 1x wash solution with a 2 min soak for each wash.
- 90 µl of TMB substrate was added to each well. The plate was covered and incubated at 37°C for 15 min.
- 50 µl of the Stop Solution was added per well.
- The optical density (OD value) of each well was read immediately using a microplate reader set to 450 nm.
- The duplicate 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 CurveExpert 1.4. A logistic equation was used for the best fit through the points on the graph.
- The CurveExpert Analyze feature was used to calculate NGAL/MMP9 concentrations of the samples based on their Average Absorbance values.

Experimental Notes

none

Figures

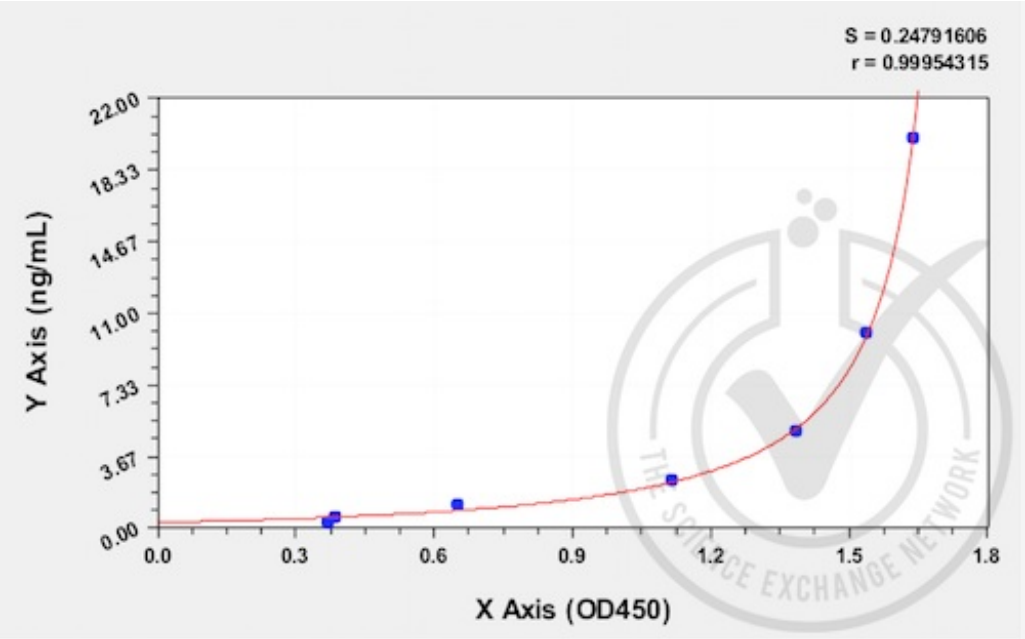

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	20	1.873	1.862	1.867	1.636	0.007311	20.006
	10	1.773	1.766	1.769	1.538	0.004773	9.9746
	5	1.619	1.610	1.615	1.384	0.006717	5.12739
	2.5	1.364	1.324	1.344	1.113	0.028314	2.46678
	1.25	0.878	0.883	0.881	0.650	0.003488	0.918345
	0.625	0.594	0.638	0.616	0.385	0.030798	0.57974
	0.312	0.570	0.629	0.600	0.369	0.041974	0.564453
	blank	0.221	0.241	0.231			
Spike Control	10	1.737	1.716	1.726	1.495	0.015	8.02297
Positive Control	Human plasma	1.082	1.119	1.101	0.870	0.0266	2.78
Negative control	Chicken Plasma	0.241	0.221	0.231	0.000	0.014	0.31158

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. NGAL/MMP9 is clearly detected in the positive sample. Spike controls indicate little interference in absorbance readings from the two-fold diluted plasma sample. Value for Average Reading is derived from the average of two readings (OD 450nm). The Average Reading for blank sample (no conjugate added) was subtracted from all Average Readings to yield Average Absorbance values for Standards, spike controls and control samples. Standard deviation is included for all samples. The concentration of samples was calculated using the Analyze feature of the CurveExpert 1.4 software for a logistic equation fit.