Validation Report #029586

Validation Date: 01/30/14

Summary

Antigen	human Matrix Metalloproteinase 9 (Gelatinase B, 92 kDa Gelatinase, 92 kDa Type IV Collagenase) (MMP9)
Catalog number	ABIN818177
Supplier	Cusabio
Supplier catalog number	CSB-E08006h
Lot number	T23095910
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies
Validation number	29586
Positive Control	Human individual postmenopausal female serum
Negative Control	Chicken plasma
Notes	Signal was detected in positive control but not in negative control samples.

Full Methods

Primary Antibody

- Antigen: human Matrix Metalloproteinase 9 (Gelatinase B, 92 kDa Gelatinase, 92 kDa Type IV Collagenase) (MMP9)
- Catalog number: ABIN818177
- 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 matrix metalloproteinase 9 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 linear equation was used for the best fit through the points on the graph.
- The CurveExpert Analyze feature was used to calculate human MMP9 concentrations of the samples based on their Average Absorbance values.

Experimental Notes

- Undiluted human serum produced artificially low concentration readings. 2-fold, 4-fold and 8-fold diluted samples showed essentially identical concentration readings.

Isotype Control Antibody

Secondary Antibody

Additional Information

Figures

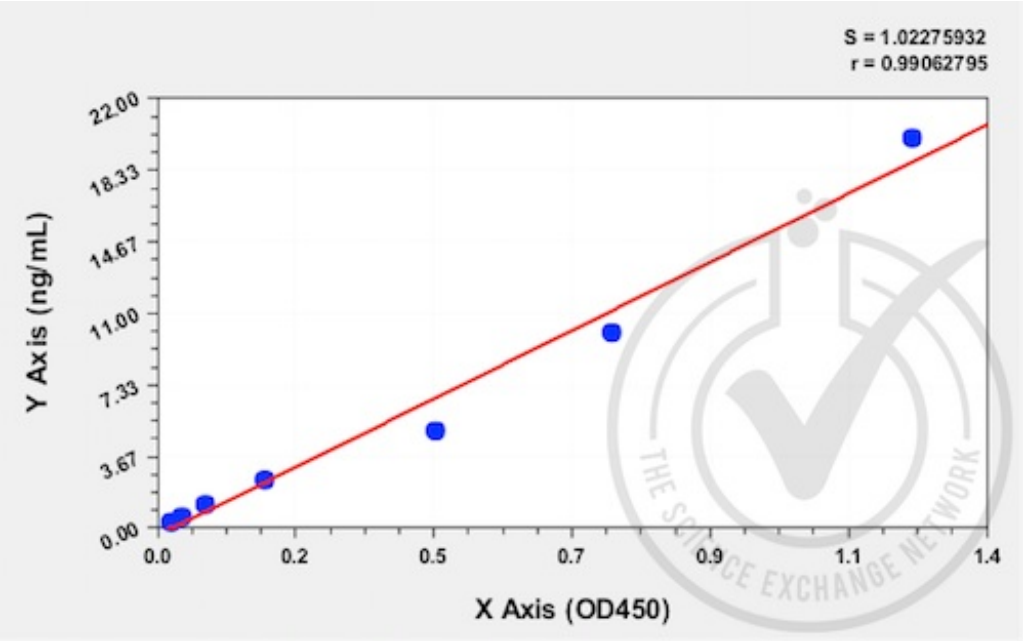

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	20	1.323	1.282	1.303	1.229	0.029	18.8
	10	0.799	0.826	0.813	0.739	0.019	11.1
	5	0.522	0.534	0.528	0.454	0.009	6.7
	2.5	0.231	0.266	0.249	0.175	0.025	2.4
	1.25	0.143	0.162	0.152	0.078	0.013	0.9
	0.625	0.117	0.113	0.115	0.041	0.002	0.3
	0.312	0.093	0.096	0.095	0.021	0.002	0.0
	blank	0.075	0.073	0.074			
Spike Control	10	0.823	0.906	0.864	0.790	0.059	12.0
Positive Control	Human serum(1/2 diluted)	0.373	0.341	0.357	0.283	0.023	8.0
Negative control	Chicken Plasma	0.079	0.102	0.090	0.016	0.016	-0.1

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. MMP9 is clearly detected in the positive sample. Spike controls indicate little interference in absorbance readings from the two-fold diluted serum 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 linear equation fit.