Validation Report #028531

Validation Date: 07/16/13

Summary

Antigen	Fibrinogen
Catalog number	ABIN673854
Lot number	980680
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories
Disclaimer*	There is a possibility that results may vary between antibody lots.
Supplier	Bioss
Supplier catalog number	bs-1240r
Validation number	28531
Positive Control	Liver
Negative Control	Skeletal muscle
Notes	Signal was detected in positive control samples but not in negative control samples.

Full Methods

Primary Antibody

- Antibody: Fibrinogen antibody
- Catalog number: ABIN673854
- Batch number: 980680

Controls

- Positive control: protein extract from human liver (specimen known to contain the target protein) was from Alamo Laboratories, Inc (Cat # 2001-TEL-Hu).
- Negative control: protein extract from mouse skeletal muscle (specimen known to not contain the target protein) was from Alamo Laboratories, Inc (Cat # 2002-TEM-Mm).
- Standard curve: serial two-fold dilutions from 200 ng/ml [200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0] were generated from Fibrinogen peptide stock diluted in 1 x 50 mM Carbonate buffer, pH 9.5.
- Spike control: standard diluted in protein lysate buffer [25 and 0].

Protocol

- A 96-well MICROLON® 600 96W High Binding 12 x 8 Clear Strip Microplate was coated with antigen by pipetting 100 µL of sample per well. All samples and standards were assayed in triplicate.
- The microplate was covered and incubated at 25°C overnight.
- After overnight coating, plate contents were discarded and wells were washed 3 times with 150 µL of 1 x PBST (PBS, 0.1% Tween-20) per well.
- Wells were then blocked with 250 µL of 1 x PBS / 0.05% Tween-20 / 1% BSA per well and incubated at 37°C for 2 h.
- After blocking, plate contents were discarded and wells were washed 3 times with 250 µL of 1 x PBS per well.
- 100 µL of primary antibody diluted 1:500 in 1 x PBS / 0.05% Tween-20 / 1% BSA was added per well and incubated at 37°C for 2 h.
- Plate contents were discarded and wells were washed 3 times with 200 µL of 1 x PBST per well.
- 100 µL of HRP conjugated secondary antibody diluted 1:10000 in 1 x PBS / 0.05% Tween-20 / 1% BSA was added per well and incubated at 37°C for 1 h.
- Plate contents were discarded and wells were washed 3 times with 200 µL of 1 x PBST per well.
- 100 µL of TMB (3,3', 5,5"-tetramethylbenzidine) substrate was added per well and incubated at 37°C for 10 min.
- 100 µL of STOP solution was added per 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. 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 y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation X = (Y-0.0122) / 0.0113.
- The equation X = (Y-0.0122) / 0.0113 was used to calculate fibrinogen concentrations of the samples based on their Average Absorbance values.

Experimental Notes

None

Figures

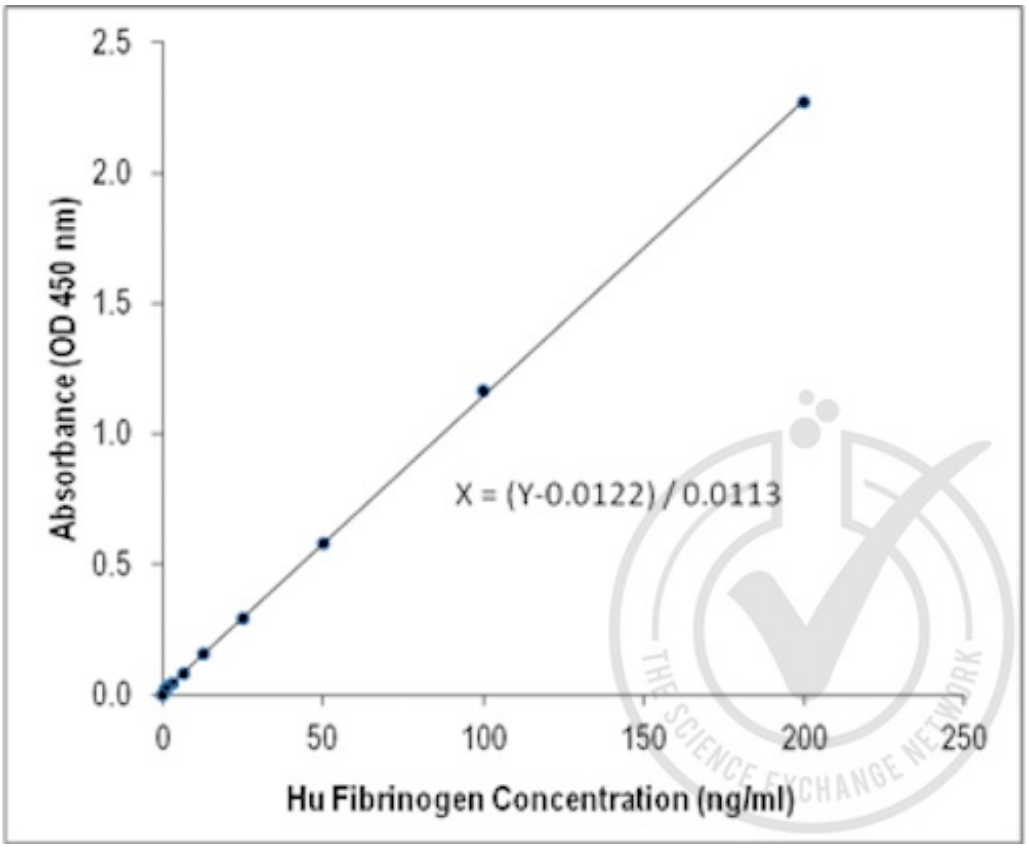

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Reading-3	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	200 ng/ml	2.3472	2.3882	2.3907	2.3754	2.2700	0.0244	199.8083
	100 ng/ml	1.2662	1.2688	1.2730	1.2693	1.1640	0.0034	101.9322
	50 ng/ml	0.6780	0.7050	0.6680	0.6837	0.5784	0.0191	50.1032
	25 ng/ml	0.3920	0.4180	0.3830	0.3977	0.2924	0.0182	24.7935
	12.5 ng/ml	0.3310	0.2650	0.2020	0.2660	0.1607	0.0645	13.1416
	6.25 ng/ml	0.1848	0.1970	0.1850	0.1889	0.0836	0.0070	6.3215
	3.125 ng/ml	0.1510	0.1490	0.1430	0.1477	0.0424	0.0042	2.6696
	1.5625 ng/ml	0.1350	0.1384	0.1368	0.1367	0.0314	0.0017	1.7021
	0.7813 ng/ml	0.1288	0.1286	0.1298	0.1291	0.0238	0.0006	1.0236
	0 ng/ml	0.1030	0.0970	0.1160	0.1053	0.0000	0.0097	-1.0767
Spike Controls	25 ng/ml	0.4010	0.3980	0.4110	0.4033	0.2980	0.0068	25.2950
	0 ng/ml	0.0980	0.1010	0.1223	0.1071	0.0018	0.0132	-0.9204
Positive Control	Liver Extract	1.9970	1.9240	2.1240	2.0150	1.9097	0.1012	167.9204
Negative Control	Muscle	0.1210	0.1060	0.0988	0.1086	0.0033	0.0113	-0.7876

Table 1: ELISA. Fibrinogen is present in the positive control sample (liver) and absent from the negative control (skeletal muscle) sample. Spike controls indicate no interference in absorbance readings from the protein lysate buffer used to prepare the positive and negative control samples. Absorbance readings (OD 450 nm) are shown for standard curve, spike controls and unknown positive (liver extract) and negative (skeletal muscle extract) control samples. Value for Average Reading is derived from the average of three 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 positive (liver extract) and negative (skeletal muscle extract) control samples. Standard deviation is included for all samples. An equation (X = (Y - 0.0122) / 0.0113) was generated from the standard curve and used to calculate fibrinogen concentrations shown in the Table.