Validation Report #029643

Validation Date: 03/27/14

Summary

Antigen	Immunoglobulin Y
Catalog number	ABIN1563240
Supplier	Elabscience
Supplier catalog number	e-el-ch0001
Lot number	AK0014FEB13014
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories
Validation number	029643
Positive Control	Chicken serum
Negative Control	Rat serum
Notes	Strong signal was observed in positive control sample, but not in negative control sample

Full Methods

Primary Antibody

- Antibody: Immunoglobulin Y
- Catalog number: ABIN1563240
- Supplier: Elabscience
- Supplier catalog number: e-el-ch-0001
- Lot number: AK0014FEB13014

Controls

- Positive control: Serum from normal adult chicken (specimen known to contain the target protein).
- Negative control: Serum from rat (specimens known to not contain the target protein).
- Standard curve: Serial two-fold dilutions from 50 ng/ml [50, 20, 12.5, 6.25, 3.13, 1.56, 0.78, 0] were generated from the standard provided in the kit using standard/sample diluent buffer.
- Spike control: Standard diluted in “Reference Standard and Sample Diluent” [6.25 and 0 ng/ml].

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 90 min at 37°C. Unbound material was aspirated but the wells were NOT washed.
- 100 µL of Biotin-Antibody (diluted 1:100 in “Biotinated Detection Ab Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 h 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 and blotted against clean absorbent paper to remove any remaining liquid.
- 100 µL of HRP- Conjugate (diluted 1:100 in “HRP-Conjugate Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 30 min 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 15 min at 37°C in the dark.
- After 15 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.
- Triplicate readings for each sample were averaged and the average zero standard optical density subtracted to yield ‘corrected absorbance at 450 nm’. The mean OD values for each standard were plotted on the X-axis against the concentration on the Y-axis using Excel. A standard curve was generated by regression analysis with four-parameter logistic.
- An equation ($y = -1.4723x4 + 6.2971x3 - 2.575x2 + 20.357x + 0.09$) was derived from the standard curve and used to calculate Immunoglobulin-Y concentrations in samples based on their Average Absorbance values.

Experimental Notes

- No experimental challenges noted

Figures

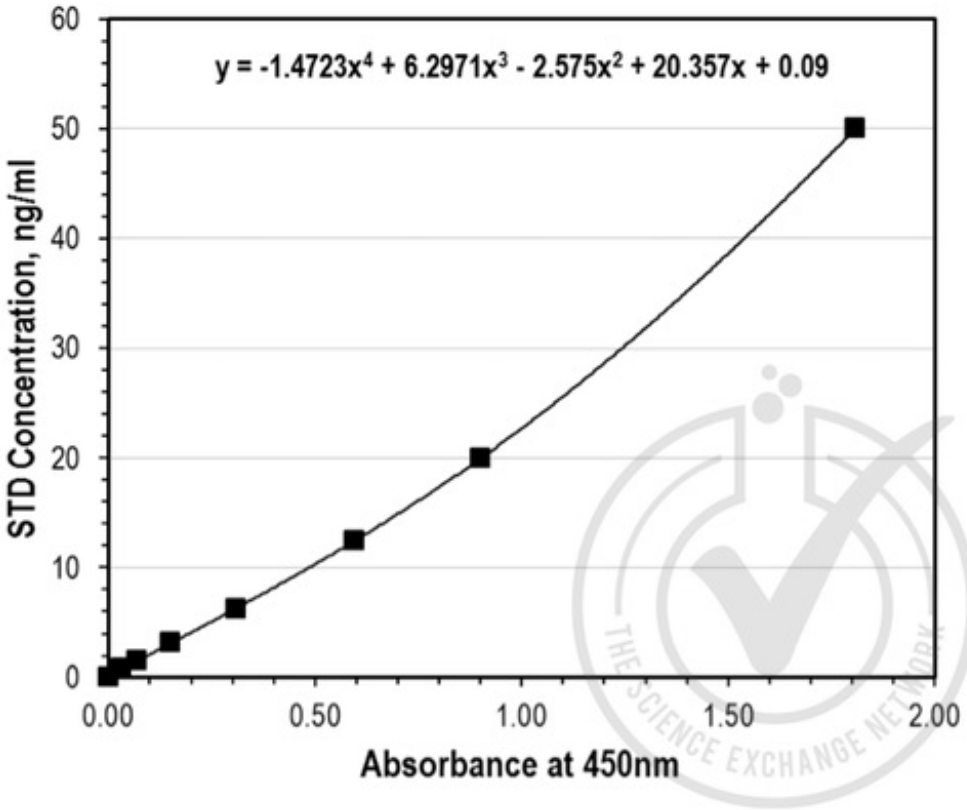

Figure 1: Graph of corrected OD 450 nm plotted for standard curve samples.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD_{450nm}	SD	Calculated conc ng/ml
		1	2	3				
Standards	50	1.884	1.806	1.863	1.851	1.809	0.033	50.00
	20	0.949	0.945	0.939	0.944	0.902	0.004	20.01
	12.5	0.630	0.644	0.640	0.638	0.596	0.006	12.46
	6.25	0.359	0.348	0.349	0.352	0.310	0.005	6.33
	3.13	0.187	0.198	0.195	0.193	0.151	0.005	3.13
	1.56	0.110	0.116	0.103	0.110	0.068	0.005	1.46
	0.78	0.073	0.079	0.071	0.074	0.032	0.003	0.75
	0	0.044	0.040	0.042	0.042	0.000	0.002	0.09
Spike Controls	0.00	0.048	0.039	0.037	0.041	-0.001	0.005	-0.08
	6.25	0.365	0.301	0.363	0.343	0.301	0.030	6.14
Test Samples	Chicken Srum 1:200K Dilution	1.281	1.271	1.266	1.273	1.231	0.006	**29.60**
	Chicken Srum 1:100K dilution	3.834	3.766	3.865	3.822	3.780	0.041	79.79
	Rat Serum 1:100 Undiluted	0.168	0.175	0.159	0.167	0.125	0.007	**2.61**
	Rat Serum 1:200K Diluted	0.044	0.038	0.036	0.039	-0.003	0.003	**-0.04**

(A) Conc of IgY in Rat Serum (-ve Control) : - 0.04 ng/ml x 200000 (dilution factor)= Undetectable
(B) Conc of IgY in Chicken Serum (+ ve Control): 29.60 ng/ml x 200000 (dilution factor) = 5.92 mg/ml
(C) Conc of IgY in Chicken Serum (+ ve Control): 79.79 ng/ml x 100000 (dilution factor) = 7.98 mg/ml *-(Out of measurement range)*
(D) Conc of IgY in Rat Serum (-ve Control): 2.61 ng/ml x 100 (dilution factor)= 0.00026 mg/ml - (*not significant).*

Table 1: Absorbance readings (OD 450 nm) 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 = -1.4723x4 + 6.2971x3 - 2.575x2 + 20.357x + 0.09) was derived from the standard curve and used to calculate Immunoglobulin-Y concentrations shown in Table 1.