Validation Report #029687

Validation Date: 04/24/14

Summary

Antigen	Cotinine
Catalog number	ABIN1721161
Supplier	Calbiotech
Supplier catalog number	CO096D-100
Lot number	COT4179
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies, Inc
Validation number	029687
Positive Control	Female rat serum spiked with cotinine
Negative Control	Unspiked female rat serum
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody

- Antigen: Cotinine
- Catalog number: ABIN1721161
- Supplier: Calbiotech
- Supplier catalog number: CO096D-100
- Lot number: COT4179

Controls

- Positive control: Rat individual female serum (Biochemed, S128927) diluted 4-fold and then spiked with 50 ng/mL cotinine (Sigma, Cat#C5923).
- Standard curve: 0, 5, 10, 25, 50 and 100 ng/mL cotinine provided in the ELISA kit
- Spike control: identical to the positive control

Protocol

- 10 µL of standard and samples were added to the 96-well strip plates provided in the kit and mixed with working solution of enzyme conjugate. All samples and standards were assayed in duplicate.
- The microplate was covered and incubated at RT for 60 min.
- Content of the wells was discarded and wells were washed 6 times with 250 µL of water.
- 100 µL of substrate was added to each well. The plate was covered and incubated at RT for 30 min in the dark.
- 100 µL of the Stop Solution was added per well.
- The entire sample was transferred into a 96-well plate (Nunc, Maxisorp)
- 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 Kaleidagraph. The concentration of samples was calculated using the CurveExpert v 1.4 logistic fit (Concentration = 3.52/(1-e-6.8*OD450).

Experimental Notes

Nothing noted.

Figures

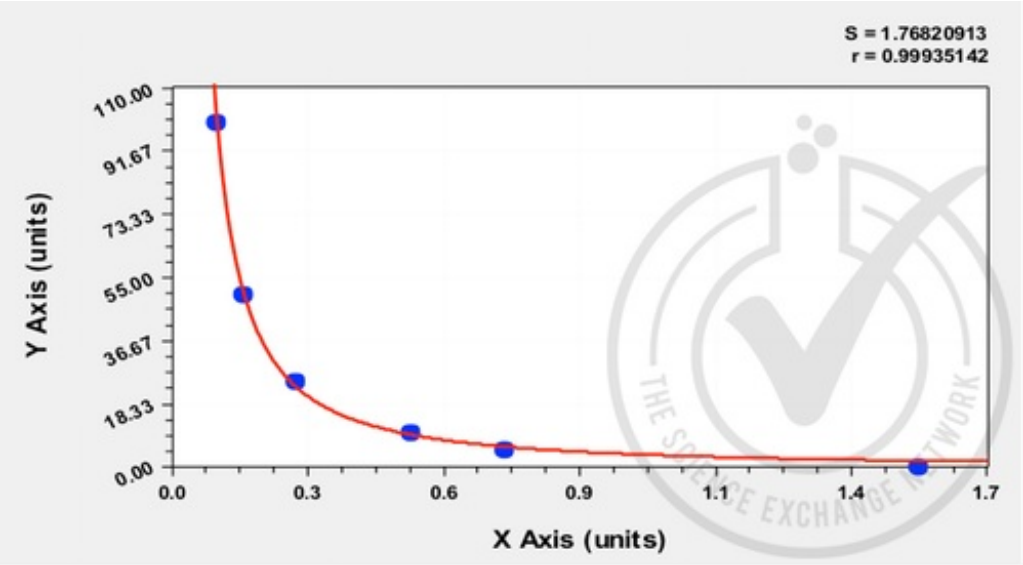

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Concentration (ng/mL)
Standard Curve	100	0.153	0.130	0.142	0.102	0.015749	100.3
	50	0.202	0.190	0.196	0.156	0.008398	48.9
	25	0.309	0.302	0.306	0.266	0.005002	24.0
	10	0.606	0.481	0.544	0.504	0.088228	11.4
	5	0.825	0.649	0.737	0.697	0.12411	8.00
	0	1.578	1.601	1.590	1.550	0.016369	3.5
Positive control	50	0.239	0.177	0.208	0.168	0.044	43.9
Negative Control	rat serum (diluted 4-fold)	1.927	1.923	1.925	1.885	0.003074	2.9

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. 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 CurveExpert v 1.4 logistic fit (Concentration = 3.52/(1-e-6.8*OD450).