Validation Report #029511

Validation Date: 12/10/13

Summary

Antigen	Thyroid Stimulating Hormone (TSH)
Catalog number	ABIN510312
Supplier	Blue Gene
Supplier catalog number	E01T0022
Lot number	20131112
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies Inc
Validation number	29511
Positive Control	Human plasma (Sigma-Aldrich, p9523)
Negative Control	Chicken plasma (Sigma-Aldrich, p3266)
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody

- Antigen: Thyroid Stimulating Hormone (TSH)
- Catalog number: ABIN510312
- Supplier: Blue Gene
- Supplier catalog number: E01T0022
- Lot number: 20131112

Controls

- Positive control: Human plasma (Sigma-Aldrich, p9523) - reconstituted at 1 mg/mL
- Negative control: Chicken plasma (Sigma-Aldrich, p3266) – reconstituted at 1 mg/mL
- Standard curve: 0, 5, 10, 25, 50, 100 mIU/mL TSH provided in the ELISA kit
- Spike control: 100 mIU/mL standard premixed with chicken plasma in a 1:1 ratio which should yield an expected value of around 50 mIU/mL

Protocol

- 50 µL of standard and samples were added 96-well strip plates provided in the kit. All samples and standards were assayed in duplicate.
- 100 µL of HRP conjugate was added and contents in the wells were mixed. The conjugate was not added to the blank sample.
- The microplate was covered and incubated at 37°C for 1 hr.
- Plate contents were discarded and wells were washed 5 times with 350 µL of 1x wash solution.
- 100 µL of premixed 1:1 substrate A and substrate B were added to each well. The plate was covered and incubated at 37°C for 10 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 Kaleidagraph. A line of best fit through the points on the graph was used to generate the equation concentration=OD*101.6-1.2.
- The equation concentration=OD*101.6-1.2was used to calculate TSH concentrations of the samples based on their Average Absorbance values.

Experimental Notes

None

Figures

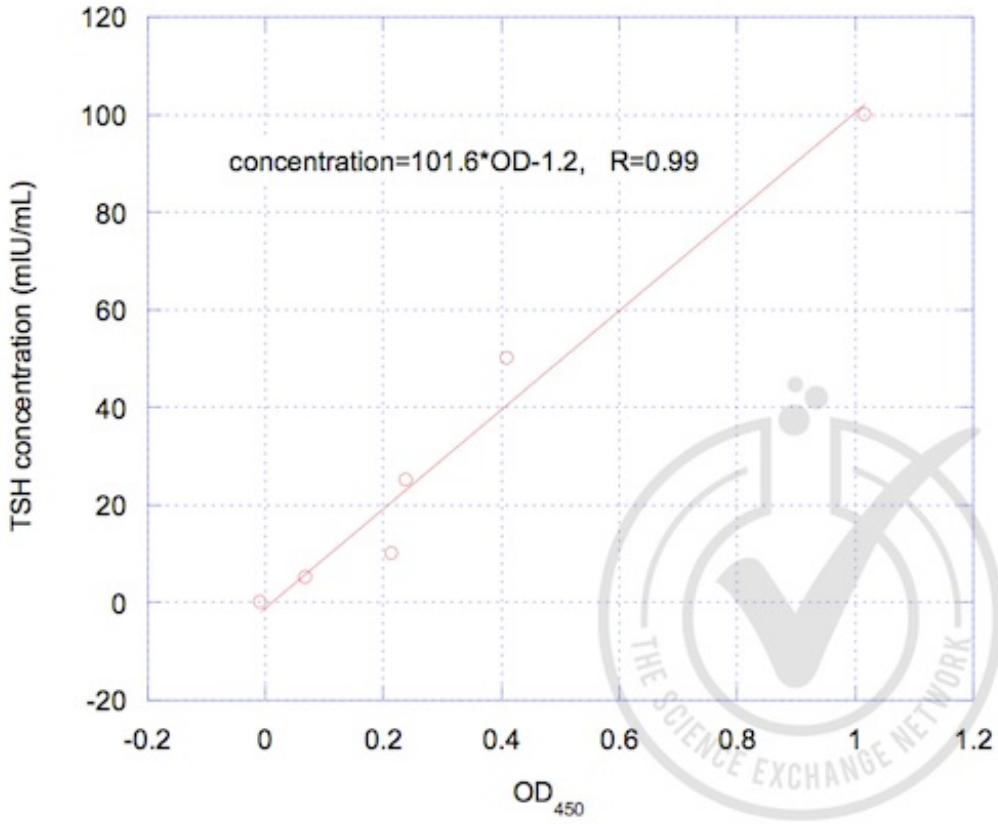

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample mIU/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	0	0.041	0.041	0.041	-0.008	0.001936	-1.9
	5	0.102	0.111	0.107	0.069	0.006569	5.9
	10	0.208	0.221	0.214	0.214	0.00969	20.7
	25	0.202	0.276	0.239	0.239	0.052635	23.2
	50	0.334	0.487	0.410	0.410	0.10808	40.6
	100	1.062	0.955	1.008	1.016	0.077505	102.2
	blank	0.041	0.041	0.041			
Spike Control	50	0.495	0.438	0.467	0.427	0.040305	42.2
Positive Control	Human plasma	0.076	0.074	0.075	0.035	0.001739	2.5
Negative control	Chicken Plasma	0.040	0.040	0.04	0.000	0.000	-1.1

Table 1: ELISA. TSH is clearly detected in the positive sample but the level of TSH in the pooled human plasma at a level of ~ 2 mIU/mL. The controls were selected on the basis of literature values of average plasma TSH concentration. Spike controls indicate some interference in absorbance readings from the two-fold diluted plasma sample. Absorbance readings (OD 450 nm) are shown 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. An equation (concentration=101.6*OD-1.2) was generated from the linear fit to the results for the standard curve and

used to calculate TSH concentrations shown in Table 1.