Validation Report #029587

Validation Date: 01/28/14

Summary

Antigen	human Luteinizing hormone (LH)
Catalog number	ABIN512262
Supplier	Blue Gene Biotech
Supplier catalog number	E01L0021
Lot number	20131113
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies Inc
Validation number	29587
Positive Control	Human individual postmenopausal female serum
Negative Control	Chicken plasma
Notes	Signal was detected in the positive control sample and not in the negative control sample.

Full Methods

Primary Antibody

- Antigen: human luteinizing hormone (LH)
- Catalog number: ABIN512262
- Supplier: Blue Gene Biotech
- Supplier catalog number: E01L0021
- Lot number: 20131113

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, 5, 10, 25, 50, 100 and 200 mIU/mL LH provided in the ELISA kit
- Spike control: 100 mIU/mL standard premixed with chicken plasma in a 1:1 ratio

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 CurveExpert 1.4. A logistic fit through the points on the graph was used to calculate concentrations.
- The Analyze feature of CurveFit expert was used to calculate LH concentrations of the samples based on their Average Absorbance values.

Experimental Notes

The OD reading for this kit was reproducibly (performed three times) very low.

Figures

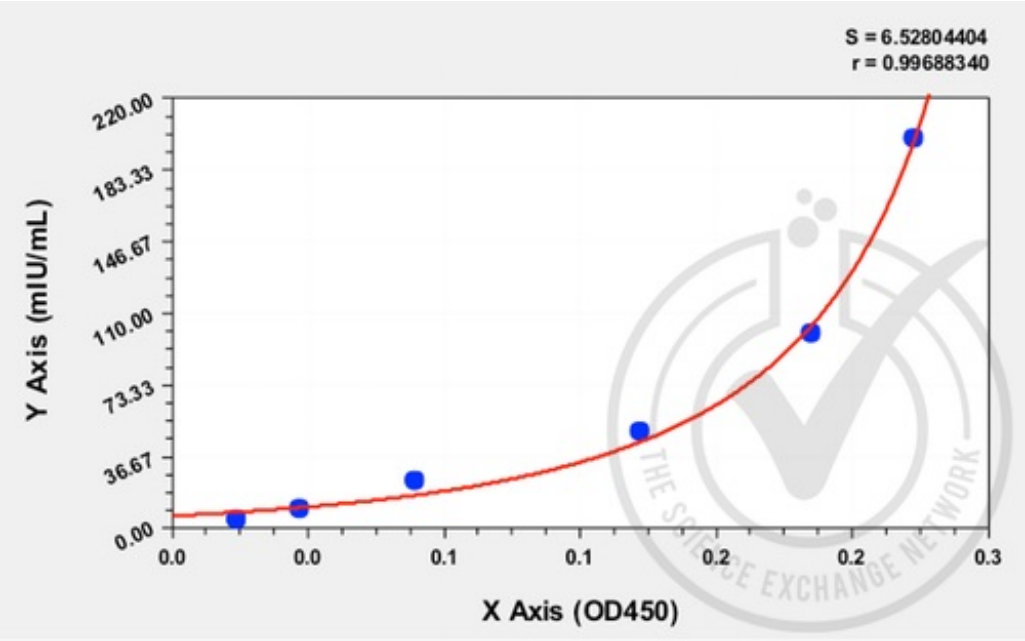

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample mIU/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	0	0.048	0.048	0.048	0.000	0.000342	7.5
	5	0.070	0.072	0.071	0.023	0.001526	9.4
	10	0.091	0.097	0.094	0.046	0.004165	11.5
	25	0.130	0.141	0.135	0.087	0.008297	18.1
	50	0.209	0.223	0.216	0.168	0.009426	45. 0
	100	0.287	0.269	0.278	0.230	0.013399	103.5
	200	0.318	0.312	0.315	0.267	0.004586	199.9
	blank	0.047	0.048	0.048			
Spike Control	50	0.215	0.231	0.223	0.175	0.011354	48.3
Positive Control	Human serum (1/2 dilute)	0.162	0.166	0.164	0.116	0.002498	47.4
Negative control	Chicken Plasma	0.049	0.048	0.048	0.000	0.001132	7.5

Table 1: ELISA. LH is clearly detected in the positive sample serum at a level of 47.4 mIU/mL. The controls were selected on the basis of literature values of plasma LH concentration. Spike controls indicate no interference in absorbance readings from the two-fold diluted plasma 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 values were generated from the logistic fit to the results (CurveExpert 1.4) for the standard curve and used to calculate LH concentrations shown in the Table.