Validation Report #029588

Validation Date: 02/01/14

Summary

Antigen	human Follicle-Stimulating Hormone (FSH)
Catalog number	ABIN512884
Supplier	Blue Gene Biotech
Supplier catalog number	E01F0001
Lot number	20131111
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies Inc
Validation number	29588
Positive Control	Human individual postmenopausal female serum
Negative Control	Chicken plasma
Notes	Signal was clearly detected in positive control sample and not in negative control sample

Full Methods

Primary Antibody

- Antigen: human Follicle-Stimulating Hormone (FSH)
- Catalog number: ABIN512884
- Supplier: Blue Gene Biotech
- Supplier catalog number: E01F0001
- Lot number: 20131111

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 mIU/mL FSH 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 line of the logistic equation fit through the points on the graph was used to calculate the FSH concentrations.

Experimental Notes

None

Isotype Control Antibody

Secondary Antibody

Additional Information

Figures

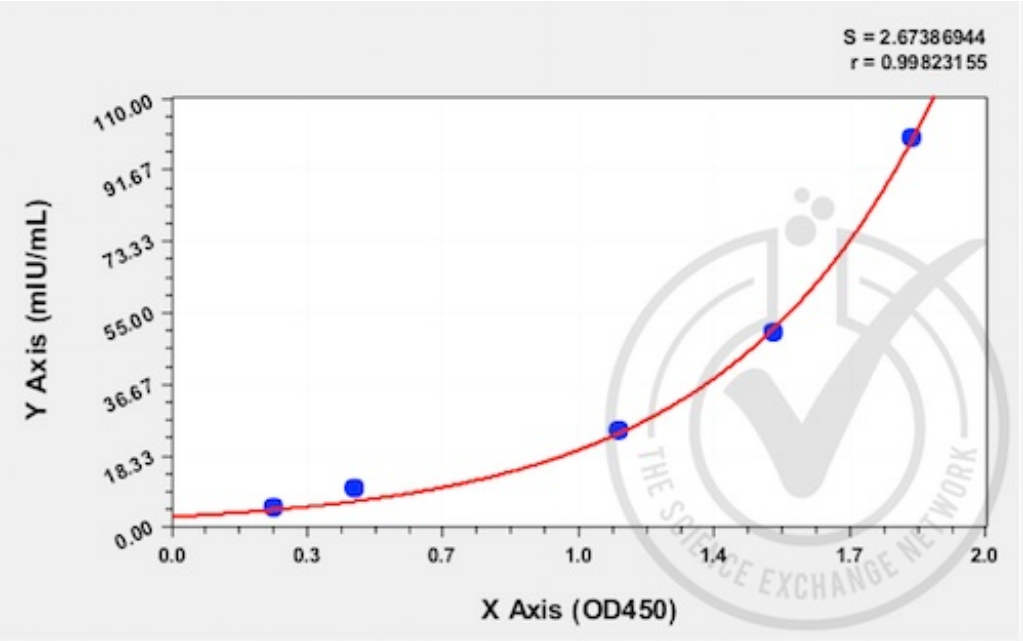

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample mIU/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	0	0.057	0.060	0.058	0.000	0.002	2.8
	5	0.317	0.309	0.313	0.255	0.005	4.6
	10	0.512	0.513	0.513	0.455	0.001	6.7
	25	1.225	1.129	1.177	1.119	0.068	24.1
	50	1.537	1.596	1.566	1.508	0.041	51.1
	100	1.913	1.913	1.913	1.855	0.000	99.7
	blank	0.058	0.059	0.059			
Spike Control	50	1.667	1.710	1.688	1.630	0.000	64.6
Positive Control	Human serum (1/2 diluted)	0.773	0.758	0.766	0.708	0.001	22.8
Negative control	Chicken Plasma	0.062	0.057	0.059	0.001	0.000	2.8

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. A logistic equation fit was generated to the results for the standard curve was generated with CurveExpert 1.4 and used to calculate FSH concentrations shown in the Table. FSH is clearly detectable at >20 mIU/mL in the positive control sample. The controls were selected on the basis of literature values of average plasma FSH concentration. Spike controls indicate some interference in absorbance readings from the two-fold diluted plasma sample.