Science EXCHANGE
555 Bryant Street, #939, Palo Alto, CA 94301-1704

Validation Report

Report #029854 | Validated On: **04/10/16**

Summary

Antigen	Human luteinizing hormone (LH)
Catalog number	ABIN365639
Supplier	Cusabio
Supplier catalog number	CSB-E12690h
Lot number	C4621151803
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies, Inc
Validation number	29854
Positive Control	Human postmenopausal individual serum (Biochemed, Lot#BC033016HSPMG)
Negative Control	Chicken serum (Biochemed, Lot#BC03316CSPMG)
Notes	Human luteinizing hormone (LH) was readily detected in the positive controls (up to 4-fold dilutions). Lower dilutions of the positive control gave lower reading of LH. Spike showed fairly poor recovery (~58%) indicating presence of inhibitory components in serum (chicken) even when it was 4-fold diluted, which is consistent with the lower readings of LH in lower dilutions of human serum.

Full Methods

ELISA kit
- Antigen: Human luteinizing hormone (LH)
- Catalog number: ABIN365639
- Supplier: Cusabio
- Supplier catalog number: CSB-E12690h
- Lot number: C4621151803

Controls
- Positive control: Human postmenopausal female serum (Biochemed, Lot#BC033016HSPMG)
- Negative control: Chicken serum (Biochemed, Lot#BC03316CSPMG)
- Standard curve: 0, 2, 5, 20, 40, 75 pIU/mL LH provided in the ELISA kit
- Spike control: 75 pIU/mL standard premixed with 4-fold diluted chicken serum in a 1:1 ratio. Lower dilutions of chicken serum produced lower readings.

Protocol
50 µL of standard and samples were added to 96-well strip plates provided in the kit with 50 µL of HRP conjugate. All samples and standards were assayed in duplicate.
The microplate was covered and incubated at 37°C for 1 hr.
Content of the wells was discarded and wells were washed 3 times with 200 µl of washing solution.
50 µl of Substrate A and Substrate B each was added to each well. The plate was covered and incubated at 37°C for 15 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 optical density (OD) value for each standard on the X-axis against the concentration on the Y-axis using CurveExpert 1.4 (CUSABIO). A logistic equation was used for the best fit through the points on the graph.
The CurveExpert Analyze feature was used to calculate human LH concentrations of the samples based on their Average Absorbance values.
Experimental Notes
- The concentration of human LH in human and chicken sera was measured according to the manufacturer's directions.

Figures

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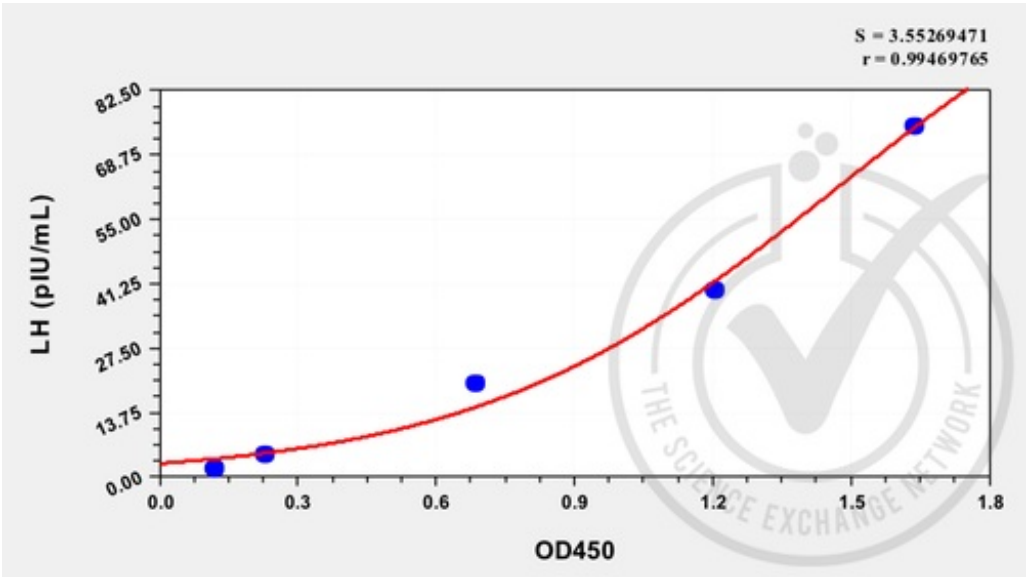

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample pIU/mL	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	75	1.708	1.717	1.712	1.674	0.006643	75
	40	1.260	1.283	1.271	1.233	0.015765	42
	20	0.710	0.765	0.738	0.700	0.039154	15
	5	0.281	0.259	0.270	0.232	0.015057	5
	2	0.165	0.156	0.160	0.122	0.006347	4
	0	0.039	0.037	0.038	0.000	0.001497	3
Spike Control	37.5	0.918	0.917	0.917	0.879	0.000419	22
Positive Control	Human serum	1.535	1.557	1.546	1.508	0.015995	62
	Human serum (2-fold dilution)	1.207	1.268	1.237	1.199	0.043212	80
	Human serum (4-fold dilution)	0.910	0.966	0.938	0.900	0.039789	92
Negative control	Chicken serum (4-fold dilution)	0.047	0.051	0.049	0.011	0.002188	12

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, spike controls, negative (chicken serum) and positive (human serum). Value for Average Reading was 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 was included for all samples. The concentration of samples was calculated using the Analyze feature of the CurveExpert 1.4 software for a logistic equation fit (Logistic Model: y=a/(1+b*exp(-cx)), a =1.2E+2, b =4.1E+1, c =2.5).