Validation Report #029781

Validation Date: 07/29/14

Summary

Antigen	Human alpha-Fetoprotein (AFP)
Catalog number	ABIN1113328
Supplier	Elabscience
Supplier catalog number	E-EL-H0070
Lot number	AK0014APR22014
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Proteopath Clinical Lab
Validation number	029781
Positive Control	Human plasma - expression is 3 ng/mL
Negative Control	Rabbit plasma (non-reactive species), mouse plasma (non-reactive species)
Notes	Target protein was detected in the positive control sample (human) and not in the negative control samples (rabbit and mouse), as expected.

Full Methods

ELISA kit

- Antigen: Human alpha-Fetoprotein (AFP)
- Catalog number: ABIN1113328
- Supplier: Elabscience
- Supplier catalog number: E-EL-H0070
- Lot number: AK0014APR22014

Controls

- Positive control: Human plasma
- Negative controls: Rabbit plasma, mouse plasma

Protocol

1. All reagents were brought up to room temperature prior to use. The 1x Wash Buffer was prepared by adding 30 mL of Concentrated Wash Buffer into 750 mL of distilled/deionized water and mixing thoroughly.
2. The vial of Standard was reconstituted with 1 mL of Sample Diluent, mixed, and allowed to sit for 10 min with gentle agitation.
3. The standard curve was prepared by creating a 2-fold dilution series of seven standards (including the original undiluted vial) using Sample Diluent. Sample Diluent alone served as the 0 ng/mL standard.
4. The Biotinylated Detection Ab was prepared by diluting the concentrated stock 1:100 in Biotinylated Detection Ab Diluent.
5. The HRP Conjugate was prepared by diluting the concentrated stock 1:100 in HRP Conjugate Diluent.
6. 100 µL of each standard and sample were added per well to the ELISA plate in duplicate and mixed gently. The plate was covered with the sealer provided and incubated for 90 min at 37 °C.
7. The liquid from each well was removed.
8. 100 µL of Biotinylated Detection Ab working solution was added to each well, and the plate was covered with sealer, gently mixed, and incubated for 1 hour at 37 °C.
9. Each well was aspirated and washed, repeating the process two times for a total of three washes. Each well was washed by filling each well with 350 µL of 1x Wash Buffer and letting it stand for 2 minutes. After the last wash, remaining Wash Buffer was removed and the plate was inverted and blotted against clean, absorbent paper towels.
10. 100 µL of HRP conjugate working solution was added to each well, the plate was covered with sealer, and incubated for 30 min at 37 °C.
11. The aspiration/wash procedure from Step 9 was repeated for an additional 5 washes.
12. 90 µL of Substrate Solution was added to each well and the plate was covered with a new plate sealer. The plate was protected from light and incubated for 15 min at 37 °C, with periodic checking to prevent overdevelopment.
13. 50 µL of Stop Solution was added to each well and mixed thoroughly. The optical density (OD) of each well was measured immediately using a microplate reader set to 450 nm.
14. The duplicate readings for each standard and samples were averaged, and the average zero (blank) standard optical density was subtracted from each value.
15. A standard curve was generated by plotting the blanked OD value for each standard on the y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation $x = (y-0.2183) / 0.068$.
16. The equation was used to calculate AFP concentrations of the samples based on their average blanked OD values.

Experimental Notes

Nothing to note

Figures

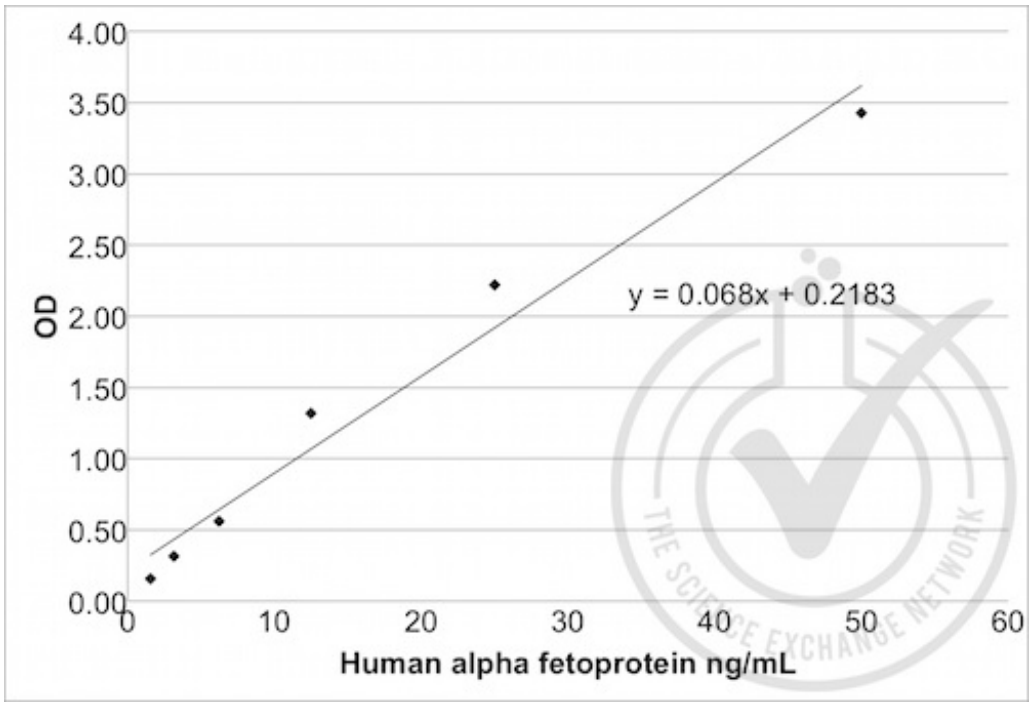

Figure 1: Human AFP standard curve graph.

	1	2	Ave OD	Ave OD - blank	Calculated fetoprotein (ng/mL)
Standard curve 0 ng/mL (blank)	0.06	0.08	0.07	0.00	
Standard curve 1.56 ng/mL	0.23	0.22	0.23	0.16	
Standard curve 3.13 ng/mL	0.38	0.39	0.39	0.32	
Standard curve 6.25 ng/mL	0.74	0.53	0.63	0.56	
Standard curve 12.5 ng/mL	1.45	1.33	1.39	1.32	
Standard curve 25 ng/mL	2.50	2.08	2.29	2.22	
Standard curve 50 ng/mL	3.55	3.44	3.50	3.43	
Positive control human plasma	0.88	0.62	0.75	0.69	6.87
Negative control rabbit plasma	0.06	0.06	0.06	-0.01	0.00
Negative control mouse plasma	0.06	0.07	0.06	-0.01	0.00

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, positive (human plasma) and negative (rabbit, mouse plasma) control samples. Value for Average Reading is derived from the average of two readings (OD 450nm). The average of the blank was subtracted from all readings and the average blanked OD readings were used to plot a standard curve. An equation x = (y - 0.2183) / 0.068 was generated from the standard curve and used to calculate alpha-Fetoprotein (AFP) concentrations shown in the Table.