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

Validation Report

Report #029856 | Validated On: **04/05/16**

Summary

Antigen	Human secreted frizzled-related protein 5 (SFRP5)
Catalog number	ABIN457072
Supplier	Cusabio
Supplier catalog number	CSB-E13427h
Lot number	V23182624
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies, Inc
Validation number	29856
Positive Control	Human mixed serum (Biochemed, Lot#BC033016HSPMG)
Negative Control	Chicken serum (Biochemed, Lot#BC03316CSPMG)
Notes	Human SFRP5 was detected in the positive samples at ~4ng/mL and was not present in the negative control. Spike controls were recovered 100%.

Full Methods

ELISA kit
- Antigen: Human secreted frizzled-related protein 5 (SFRP5)
- Catalog number: ABIN457072
- Supplier: Cusabio
- Supplier catalog number: CSB-E13427h
- Lot number: V23182624

Controls
- Positive control: Human mixed serum (Biochemed, Lot#BC033016HSPMG)
- Negative control: Chicken serum (Biochemed, Lot#BC03316CSPMG)
- Standard curve: 0, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 pg/mL human SFRP5 provided in the ELISA kit
- Spike control: 100 pg/mL standard premixed with chicken serum in a 1:1 ratio

Protocol
100 µL of standard and samples were added 96-well strip plates provided in the kit. All samples and standards were assayed in duplicate.
The microplate was covered and incubated at 37°C for 2 hr.
Plate contents were discarded.
100 µL of biotin antibody conjugate was added and incubated at 37°C for 1 hr.
Content of the wells was discarded and wells were washed 3 times with 200 µl of 1x wash solution
100 µL of HRP-avidin conjugate was added and incubated at 37°C for 1 hr.
Contents of the wells were discarded and wells were washed 5 times with 300 µl of 1x wash solution.
90 µl of TMB substrate 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 function equation was used for the best fit through the points on the graph.
The CurveExpert Analyze feature was used to calculate human SFRP5 concentrations of the samples based on their Average Absorbance values.
Experimental Notes
- The concentration of human SFRP5 in human and chicken sera was measured according to the manufacturer's directions.

Figures

Validation Report

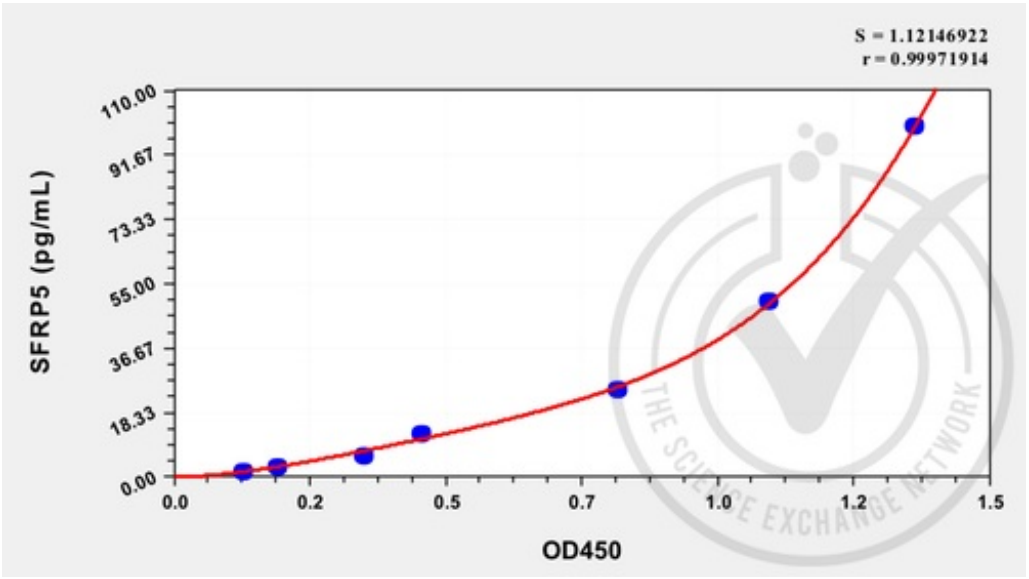

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	100.000	1.451	1.472	1.462	1.363	0.014715	99.9
	50	1.146	1.243	1.194	1.095	0.068562	50.8
	25	0.955	0.875	0.915	0.816	0.056396	24.1
	12.5	0.545	0.561	0.553	0.454	0.011098	9.00
	6.25	0.464	0.434	0.449	0.350	0.020914	6.7
	3.125	0.290	0.288	0.289	0.190	0.00149	4.32
	1.5625	0.222	0.230	0.226	0.127	0.005869	3.63
	0.000	0.08	0.098	0.085	0.000	0.001186	0.0
Spike Control	50	1.221	1.125	1.173	1.103	0.067984	52
Positive Control	Human serum(810x diluted)	0.351	0.351	0.351	0.281	0.0000	3977
Negative control	Chicken serum (810x diluted)	0.065	0.076	0.07	0.000	0.008973	2.55

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. Value for Average Reading was derived from the average of two readings (OD 450nm). The Average Reading for blank sample (no sample 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 function fit.