Validation Report #029584

Validation Date: 01/22/14

Summary

Antigen	Human Defensin, alpha 3, Neutrophil-Specific (DEFa3)
Catalog number	ABIN419782
Supplier	USCNK
Supplier catalog number	SEE135Hu
Lot number	L131225503
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Shakti Bioresearch
Validation number	29584
Positive Control	Human serum
Negative Control	Mouse serum
Notes	Minimal levels of signal detected in negative control. Percent recovery of the spiked samples shows that there is matrix interference. Dilution of >50 fold is required for accurate measurement of the analyte in human serum samples.

Full Methods

Primary Antibody

- Antigen: Defensin, alpha 3, Neutrophil-Specific (DEFa3)
- Catalog number: ABIN419782
- Supplier: USCNK
- Supplier catalog number: SEE135Hu
- Lot number: L131225503

Controls

- Positive control: normal human serum
- Negative control: mouse serum
- Standard curve: serial two-fold dilutions from 30 ng/ml [30, 15, 7.5, 3.75, 1.875, 0.9375, 0] were generated from the standard provided in the kit using standard diluent buffer.
- Spike control: standard diluted in human or mouse serum [6 ng/mL].
- Control lyophilized powder was dissolved in 150uL standard diluent buffer (7.39 ng/mL)

Protocol

- All reagents in the ELISA kit were brought up to room temperature (RT) before use.
- 100 µl of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in triplicate.
- The plate was covered with sealer (provided in kit) and incubated for 120 mins at 37°C.
- Liquid was removed from each well by pipette.
- Detection Reagent A was diluted 100 fold in Assay Diluent A. 100 µl of diluted detection reagent A was added to each well and the plate was sealed. The plate was tapped to ensure mixing and incubated for 60 mins at 37°C.
- Wells were washed with 300 µl wash buffer three times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- Detection Reagent B was diluted 100 fold in Assay Diluent B. 100 µl of diluted detection reagent B was added to each well and the plate was sealed. The plate was tapped to ensure mixing and incubated for 30 mins at 37°C.
- Wells were washed with 300 µl wash buffer five times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 90 µl of Substrate Solution was added to each well and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated at room temperature in the dark.
- After about 10 mins, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µl of Stop Solution to each well.
- The optical density (OD value) of each well was read using a micro-plate reader set to 450 nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. A standard curve was generated by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis using Softmax Pro softare.
- The equation y = (A-D)/(1 + (x/C)^B) + D was used to calculate IL-6 concentrations of the samples based on their average OD values.

Experimental Notes

- Percent recovery of the spiked samples shows that there is matrix interference. Dilution of >50 fold is required for accurate measurement of the analyte in human serum samples.

Isotype Control Antibody

Secondary Antibody

Additional Information

Figures

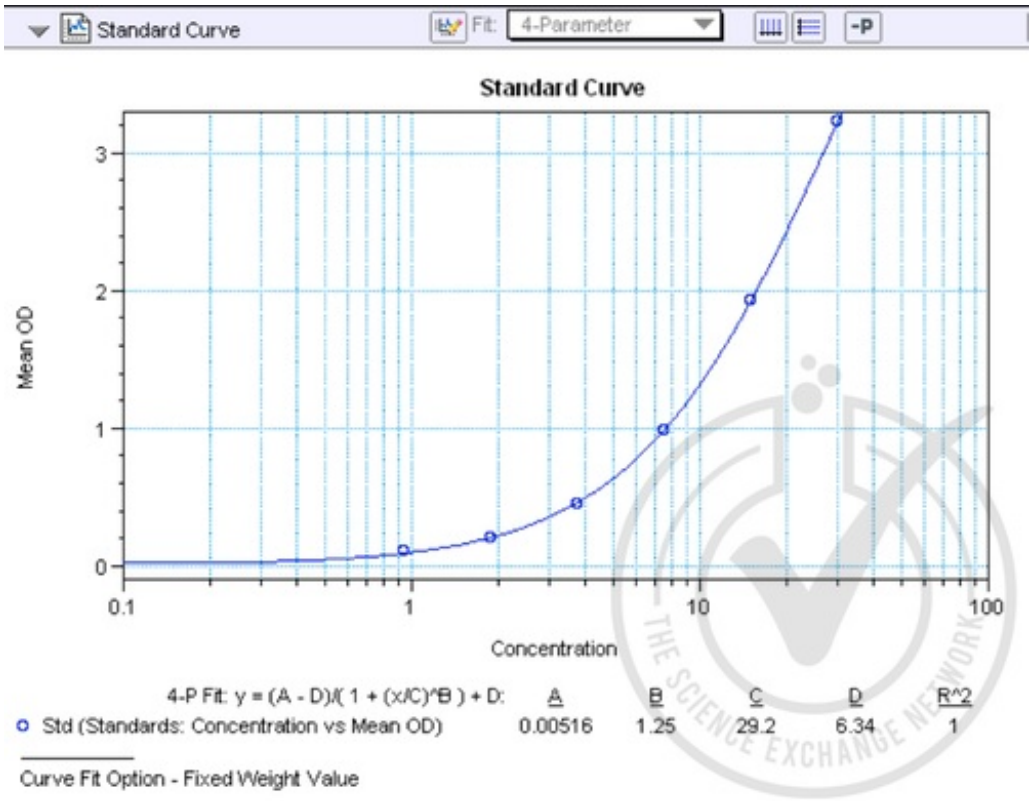

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Sample Description	OD 450nm	Conc (ng/mL)	Mean Conc (ng/mL)	Std. Dev.	CV%	Dilution Factor	Conc (ng/mL) in neat serum	% Recovery
Human serum diluted 10 fold	2.482	20.48	17.26	2.79	16.2	10	172.59	
	2.004	15.72						
	1.989	15.58						
6 ng/mL of DEFa3 spiked into diluted human serum	2.532	21.03	19.91	2.15	10.8			85.6
	2.554	21.28						
	2.185	17.43						
Human serum diluted 50 fold	0.790	6.11	5.51	0.71	12.9	50	275.55	
	0.732	5.70						
	0.594	4.73						
6 ng/mL of DEFa3 spiked into diluted human serum	1.237	9.37	8.62	0.68	7.9			74.9
	1.115	8.46						
	1.058	8.04						
Mouse serum diluted 10 fold	0.060	0.66	0.56	0.08	14.8	10	5.63	
	0.046	0.52						
	0.045	0.51						
Mouse serum diluted 50 fold	0.028	0.33	0.33	0.03	7.9	50	16.35	
	0.030	0.35						
	0.026	0.30						
100 µL of Control sample tested	0.423	3.51	3.51				35.08	474.7
30 µL of Control sample tested	0.171	1.62	1.62				53.93	729.8
15 µL of Control sample tested	0.100	1.03	1.03				68.80	931.0

Table 1: ELISA. DEFa3 could be detected in human serum (positive control) after 10 fold dilution. Spike controls indicate that there is interference from the human serum matrix and a dilution of >50 fold is required. Mouse serum was used as negative control, there were residual levels of DEFa3. Control sample concentration is expected to be 7.39 ng/mL

Sample	Concentration (ng/mL)	BackCalcConc (ng/mL)	OD 450nm	Mean OD	Std.Dev.	CV%	%Accuracy
St02	30	30.41	3.254	3.227	0.068	2.1	101.4
		30.76	3.277				102.5
		28.85	3.15				96.2
St03	15	14.46	1.864	1.927	0.083	4.3	96.4
		15.88	2.022				105.8
		14.73	1.895				98.2
St04	7.5	8.43	1.11	0.984	0.111	11.2	112.3
		7.19	0.94				95.8
		6.92	0.903				92.2
St05	3.75	4.28	0.531	0.448	0.078	17.3	114.1
		3.61	0.437				96.2
		3.18	0.377				84.7
St06	1.875	1.94	0.212	0.203	0.023	11.1	103.7
		2.01	0.22				107.0
		1.67	0.178				89.3
St07	0.938	1.20	0.12	0.106	0.014	13.1	127.9
		1.08	0.106				115.6
		0.96	0.092				102.0
St08	0	Range?	-0.001	-0.005	0.003	68.2	
		Range?	-0.005				
		Range?	-0.008				

Table 2: Value for Average Reading is derived from the average of three readings (OD 450nm). The Average Reading for BLANK (0 ng/ml) was subtracted from all Average Readings to yield Average Absorbance values for Standards. Standard deviation is included for all samples. An equation y = (A-D)/(1 + (x/C)^B) + D was generated from the standard curve and used to calculate DEFa3 concentrations shown in the Figure 1.