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

Validation Report

Report #029855 | Validated On: **04/02/16**

Summary

Antigen	Human β2-microglobulin (B2M)
Catalog number	ABIN365026
Supplier	Cusabio
Supplier catalog number	CSB-E04883h
Lot number	C738151804
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Affina Biotechnologies, Inc
Validation number	29855
Positive Control	Human pooled serum (Biochemed, Lot#BC033016HSPMG)
Negative Control	Chicken serum (Biochemed, Lot#BC03316CSPMG)
Notes	β2-microglobulin was easily detected in diluted and undiluted positive samples (up to 8-fold dilutions). Very low signal was seen in the negative control. Spike showed very good recovery (~82%).

Full Methods

ELISA kit
- Antigen: Human β2-microglobulin (B2M)
- Catalog number: ABIN365026
- Supplier: Cusabio
- Supplier catalog number: CSB-E04883h
- Lot number: C738151804

Controls
- Positive control: Human pooled serum (Biochemed, Lot#BC033016HSPMG)
- Negative control: Chicken serum (Biochemed, Lot#BC03316CSPMG)
- Standard curve: 0, 0.025, 0.1, 0.5, 2.5, 10 µg/mL β2-microglobulin provided in the ELISA kit
- Spike control: 2.5 µg/mL standard premixed with chicken serum in a 1:1 ratio

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 OD value for each standard on the x-axis against the concentration on the Y-axis using CurveExpert 1.4 (CUSABIO). An exponential equation was used for the best fit through the points on the graph.
The CurveExpert Analyze feature was used to calculate human β2-microglobulin concentrations of the samples based on their Average Absorbance values.
Experimental Notes
- The concentration of human β2-microglobulin in human and chicken sera was measured according to the manufacturer's directions.

Figures

Validation Report

Report #029855 | Validated On: **04/02/16**

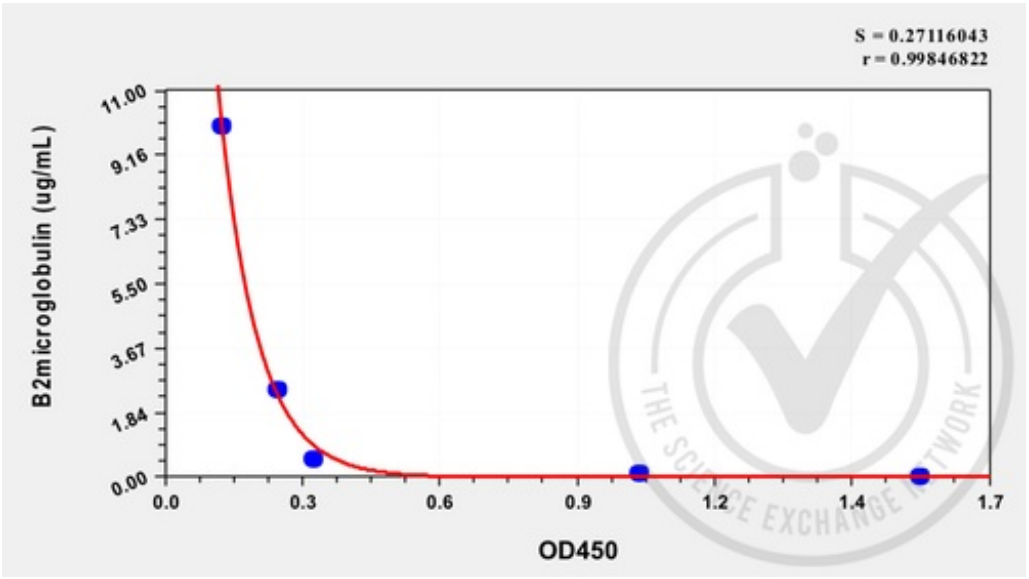

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample µg/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	10	0.181107	0.184418	0.182763	0.129763	0.002342	10.0
	2.5	0.301744	0.301003	0.301373	0.248373	0.000524	0.96
	0.5	0.351127	0.400073	0.3756	0.3226	0.034611	0.61
	0.1	1.063496	1.040647	1.052071	0.999071	0.016157	0.14
	0.025	1.613362	1.651904	1.632633	1.579633	0.027253	0.088
Spike Control	1.25	0.288	0.289	0.288	0.235	0.0004	1.06
Positive Control	Human serum	0.996	0.968	0.982	0.949	0.020225	1.25
Negative control	Chicken serum	2.100	1.918	2.008	0.008797	0.130612	0.0078

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 an exponential equation fit (Concentration = ae^(bx), a =5.1E+001, b =-1.3E+001)