Science EXCHANGE
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Validation Report

Report #029857 | Validated On: **04/29/16**

Summary

Antigen	Human Immunoglobulin G (IgG)
Catalog number	ABIN510001
Supplier	BlueGene
Supplier catalog number	NE01I0058
Lot number	HU2016ZH021
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Celplor LLC
Validation number	29857
Positive Control	Normal Human Serum (Sigma, H4522)
Negative Control	Normal Chicken Serum (Jackson ImmunoResearch, 003-000-001)
Notes	Human IgG ELISA. Linear range for standard curve is between 0 ng/mL and 25 ng/mL. 1:1000000 dilution of human serum shows the absorbance within the linear range of standard curve.

Full Methods

ELISA kit
- Antigen: Human Immunoglobulin G (IgG)
- Catalog number: ABIN510001
- Supplier: BlueGene
- Supplier catalog number: NE01I0058
- Lot number: HU2016ZH021

Controls
- Positive control: Normal Human Serum (Sigma, H4522)
- Negative control: Normal Chicken Serum (Jackson ImmunoResearch, 003-000-001)
- Normal human and chicken serum were diluted with PBS at 1:10, 1:100, 1:1000, 1:10000, 1:100000 and 1:1000000
- Standard curve: Serial two-fold dilutions of standards (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0 ng/mL) were provided in the kit

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 duplicate.
The plate was covered with sealer (provided in kit) and incubated for 1.5 hour at 37°C.
Liquid was removed from each well by pipette.
Wells were washed with 300 µL wash buffer (1X) 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.
100 uL of enzyme conjugate (1X dilution with dilution buffer provided by the kit) was added per well.
The plate was covered with sealer (provided in kit) and incubated for 1.5 hour at 37 °C.
Liquid was removed from each well by pipette.
Wells were washed with 300 µL wash buffer (1X) 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.
50 µL of Substrate A and 50 uL of Substrate B were added to each well and the plate was covered with a new plate sealer.
The plate was tapped to ensure mixing and incubated for 15 min at 37°C in the dark.
The reaction was terminated by adding 50 µL of Stop Solution to each well.
The optical density (OD value) of each well was immediately read using a micro-plate reader set to 450 nm with 570nm as reference.
A standard curve was generated by plotting the normalized 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+0.0048)/0.041.
The equation X=(Y+0.0048)/0.041 was used to calculate IgG concentrations of the samples based on their normalized average OD values.
Experimental Notes
- Only 1:1000000 dilution of human serum has the absorbance within the linear range of standard curve. Therefore at least 1:1000000 dilution is required for the accurate measurement of IgG in human serum sample with this kit.
- Weak absorbance was observed in chicken serum at 1:100000 and 1:1000000 dilutions, suggesting weak cross reaction with chicken IgG.

Figures

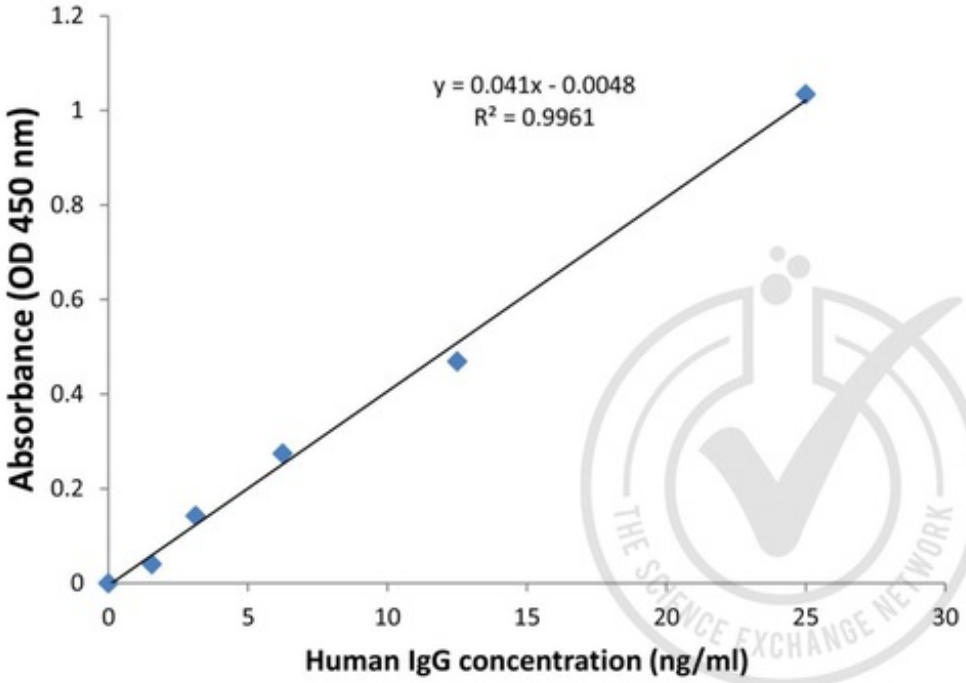

Figure 1: Graph of corrected-average absorbance (OD 450nm - OD 570nm) readings plotted for standard curve samples. Readings from 50 and 100 ng/mL standard were not included for the generation of standard curve.

Type	sample	reading1	reading2	Average	Normalized Average	Calculated Conc (ng/ml)
standard curve	100 ng/ml	2.357	2.312	2.335	2.095	N/A
	50 ng/ml	1.728	1.745	1.737	1.497	N/A
	25 ng/ml	1.312	1.236	1.274	1.034	25.34
	12.5 ng/ml	0.686	0.731	0.709	0.469	11.54
	6.25 ng/ml	0.493	0.535	0.514	0.274	6.80
	3.125 ng/ml	0.388	0.375	0.382	0.142	3.57
	1.5625 ng/ml	0.271	0.288	0.280	0.040	1.08
	0 ng/ml	0.23	0.25	0.240	0.000	0.12
PBS	PBS	0.156	0.153	0.155	-0.001	0.10
Human serum	no dilution	3.587	3.437	3.512	3.357	82.00
	1:10	3.112	3.221	3.167	3.012	73.57
	1:100	3.08	3.234	3.157	3.002	73.34
	1:1000	2.927	3.098	3.013	2.858	69.81
	1:10000	2.41	2.58	2.495	2.340	57.19
	1:100000	1.553	1.781	1.667	1.512	37.00
	1:1000000	0.571	0.438	0.505	0.350	8.64
Chicken serum	no dilution	0.14	0.148	0.144	-0.011	-0.15
	1:10	0.136	0.13	0.133	-0.022	-0.42
	1:100	0.13	0.123	0.127	-0.029	-0.58
	1:1000	0.175	0.133	0.154	-0.001	0.09
	1:10000	0.129	0.137	0.133	-0.022	-0.42
	1:100000	0.156	0.184	0.170	0.015	0.48
	1:1000000	0.19	0.19	0.190	0.035	0.97

Linear range of standard curve: 0-25 ng/ml

Human IgG in normal serum (calculated from 1:1000000 dilution): 8.64 mg/ml

Figure 2: Table of absorbance corrected readings (OD 450nm - OD570nm) for standard curve and serum samples. Value for Average Reading was derived from the average of two readings. PBS was used for the dilution of serum samples. The Average Reading for 0 ng/ml Standard was subtracted from Average Readings of other standards and the Average Reading for PBS was subtracted from Average Readings of serum samples

to yield normalized Average Absorbance values. An equation X=(Y+0.0048)/0.041 was generated from the standard curve and used to calculate IgG concentrations shown in the Table.