Validation Report #029789

Validation Date: 08/12/14

Summary

Antigen	Chemokine (C-X-C Motif) Ligand 16 (CXCL16)
Catalog number	ABIN365901
Supplier	Cusabio
Supplier catalog number	csb-e08871h
Lot number	Q03184077
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029789
Positive Control	Human serum - expression is 1.3 ng/mL
Negative Control	Goat serum (non-reactive species)
Notes	Target protein was detected in the positive control sample and not in the negative control sample as expected.

Full Methods

ELISA kit

- Antigen: Chemokine (C-X-C Motif) Ligand 16 (CXCL16)
- Catalog number: ABIN365901
- Supplier: Cusabio
- Supplier catalog number: csb-e08871h
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Controls

- Positive control: Human serum (Sigma Aldrich, Cat# H6914-20ML, Lot# SLBK2170V)
- Negative control: Goat serum (Sigma Aldrich, Cat# G9023-10ML, Lot# SLBH2670V)

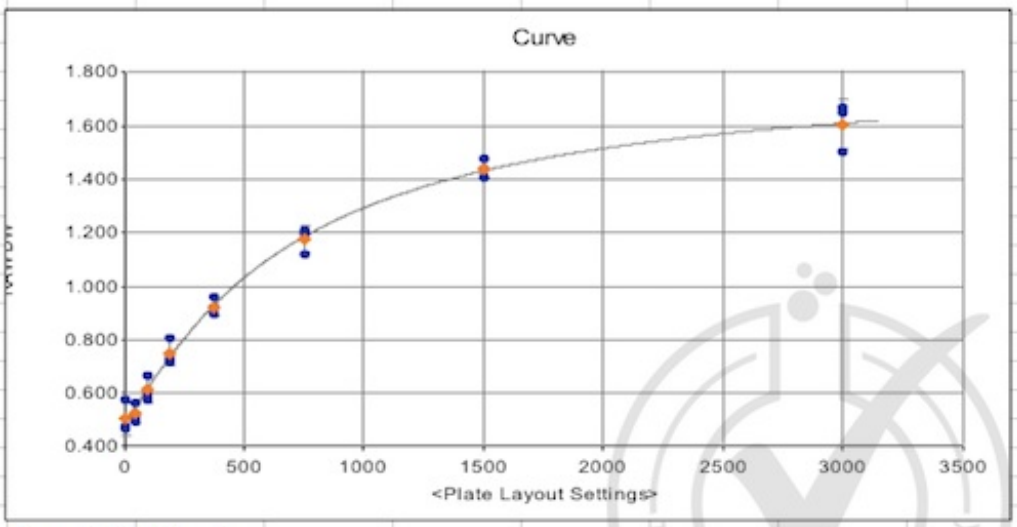
Protocol

1. All reagents were brought up to room temperature for 30 minutes prior to use. The 1x Wash Buffer was prepared by adding 20 mL of 25x Wash Buffer Concentrate to 480 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 15 minutes 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 pg/mL standard.
4. The assay plate was removed from the foil pouch and 100 µL of each standard and sample were added to the appropriate wells, in triplicate. The plate was covered with the adhesive strip provided and incubated for 2 hours at 37 °C.
5. Approximately 10 minutes before the incubation ended, a 1x Biotin-antibody solution was prepared by diluting 60 µL of 100x Biotin-antibody into 5940 µL of Biotin-antibody Diluent.
6. The liquid from each well was removed.
7. 100 µL of 1x Biotin-antibody solution was added to each well, and the plate was covered with a new adhesive strip, and incubated for 1 hour at 37 °C.
8. Approximately 10 minutes before the incubation ended, a 1x HRP-avidin solution was prepared by diluting 60 µL of 100x HRP-avidin into 5940 µL of HRP-avidin Diluent.
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 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 1x HRP-avidin solution was added to each well, the plate was covered with a new adhesive strip, and incubated for 1 hour at 37 °C.
11. The aspiration/wash procedure from Step 9 was repeated for an additional 5 washes.
12. 90 µL of TMB Substrate was added to each well. The plate was protected from light and incubated for 15-30 minutes 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 within 5 minutes using a microplate reader set to 450 nm.
14. A standard curve was generated by plotting the OD value for each standard on the y-axis against the concentration on the x-axis. A line of best fit through the points on the graph was used to generate an equation to calculate CXCL16 concentrations of the samples based on their average OD values.

Experimental Notes

The TMB substrate (Lot 03190614) used for this assay was light blue prior to addition. The 1:100 dilution of the positive control (human serum) reported much higher values than its less dilute counterparts and should be treated as outliers.

Figures



Curve Fitting Results

Curve Name	Curve Formula	A	B	C	D	R2
Curve	Y = (A-D)/(1+(X/C)^B) + D	0.493	1.16	689	1.81	0.999

Figure 1: CXCL16 standard curve graph and equation.

Layout

	1	2	3	4	5	6	
A	STD1	STD1	STD1	SPL1:1	SPL1:1	SPL1:1	Well ID
	0	0	0	2	2	2	Conc/Dil
				Human serum	Human serum	Human serum	Name
B	STD2	STD2	STD2	SPL1:2	SPL1:2	SPL1:2	Well ID
	46.88	46.88	46.88	5	5	5	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	93.75	93.75	93.75	10	10	10	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	187.5	187.5	187.5	20	20	20	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5	STD5	STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	375	375	375	40	40	40	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	750	750	750	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	1500	1500	1500	100	100	100	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	3000	3000	3000	1	1	1	Conc/Dil
				Goat serum	Goat serum	Goat serum	Name

Figure 2: Plate layout. Standard concentrations are in pg/mL; serum dilution values indicate their fold change from

the undiluted stock.

RAW DW

	1	2	3	4	5	6	
A	0.574	0.467	0.471	1.672	1.531	1.363	RAW DW
B	0.565	0.514	0.493	1.09	1.201	1.038	RAW DW
C	0.668	0.599	0.575	0.854	0.888	0.731	RAW DW
D	0.804	0.717	0.732	0.561	0.575	0.503	RAW DW
E	0.959	0.919	0.893	0.346	0.368	0.273	RAW DW
F	1.199	1.118	1.21	0.285	0.304	0.223	RAW DW
G	1.479	1.44	1.407	0.782	0.782	0.638	RAW DW
H	1.65	1.672	1.501	0.084	0.072	0.072	RAW DW

Figure 3: Raw OD readings of standards and controls.

Conc

	1	2	3	4	5	6	
A	65.891	<0.000	<0.000	>3150.000	2115.689	1215.486	Conc
B	59.187	19.968	0.558	584.216	781.085	508.755	Conc
C	136.717	84.481	66.634	297.013	331.083	187.019	Conc
D	250.046	175.558	187.845	56.201	66.634	10.617	Conc
E	408.911	363.867	336.255	<0.000	<0.000	<0.000	Conc
F	776.998	628.769	799.768	<0.000	<0.000	<0.000	Conc
G	1749.828	1536.83	1385.705	230.422	230.422	113.739	Conc
H	>3150.000	>3150.000	1891.084	<0.000	<0.000	<0.000	Conc

Conc x Dil

	1	2	3	4	5	6	
A				>6300.000	4231.378	2430.972	Conc x Dil
B				2921.082	3905.425	2543.776	Conc x Dil
C				2970.133	3310.828	1870.193	Conc x Dil
D				1124.012	1332.69	212.349	Conc x Dil
E				<0.000	<0.000	<0.000	Conc x Dil
F				<0.000	<0.000	<0.000	Conc x Dil
G				23042.16	23042.16	11373.95	Conc x Dil
H				<0.000	<0.000	<0.000	Conc x Dil

Figure 4: CXCL16 concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 2763 pg/mL (2.763 ng/mL) of CXCL16 was detected in the positive control (human serum) and 0 pg/mL of CXCL16 was detected in the negative control (goat serum). Readings from the 1:100 dilution of human serum were abnormally high and were excluded from the calculation of the average CXCL16 level in human serum.