Validation Report #029785

Validation Date: 08/12/14

Summary

Antigen	Chemokine (C-C Motif) Ligand 22 (CCL22)
Catalog number	ABIN365803
Supplier	Cusabio
Supplier catalog number	csb-e04660h
Lot number	Z02184066
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029785
Positive Control	Human serum - expression is ~250 pg/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-C Motif) Ligand 22 (CCL22)
- Catalog number: ABIN365803
- Supplier: Cusabio
- Supplier catalog number: csb-e04660h
- Lot number: Z02184066

Controls

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

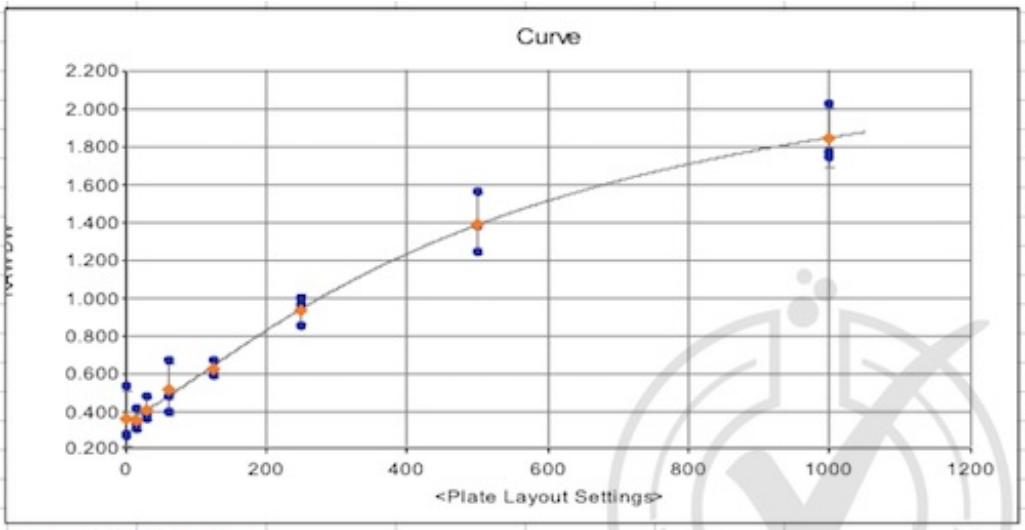
Protocol

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 CCL22 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. Other than that, there were no experimental challenges noted.

Figures



Curve Fitting Results

Curve Name	Curve Formula	A	B	C	D	R2
Curve	Y = (A-D)/(1+(X/C)^B) + D	0.355	1.29	539	2.53	0.999

Figure 1: CCL22 standard curve graph and equation.

Layout

	7	8	9	10	11	12	
A	STD1	STD1	STD1	SPL1:1	SPL1:1	SPL1:1	Well ID
	0	0	0	1	1	1	Conc/Dil
				Human serum	Human serum	Human serum	Name
B	STD2	STD2	STD2	SPL1:2	SPL1:2	SPL1:2	Well ID
	15.625	15.625	15.625	2	2	2	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	31.25	31.25	31.25	5	5	5	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	62.5	62.5	62.5	10	10	10	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5	STD5	STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	125	125	125	20	20	20	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	250	250	250	40	40	40	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	500	500	500	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	1000	1000	1000	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

	7	8	9	10	11	12	
A	0.282	0.272	0.533	0.829	1.049	0.934	RAW DW
B	0.309	0.329	0.417	0.837	0.847	0.898	RAW DW
C	0.362	0.39	0.475	0.522	0.604	0.558	RAW DW
D	0.397	0.483	0.668	0.324	0.359	0.336	RAW DW
E	0.613	0.587	0.671	0.144	0.168	0.142	RAW DW
F	0.85	0.966	0.998	0.106	0.105	0.097	RAW DW
G	1.242	1.383	1.561	0.096	0.095	0.084	RAW DW
H	1.775	1.744	2.031	0.059	0.061	0.046	RAW DW

Figure 3: Raw OD readings of standards and controls.

Conc

	7	8	9	10	11	12	
A	<0.000	<0.000	82.932	200.534	300.061	246.043	Conc
B	<0.000	<0.000	35.146	203.897	208.122	230.084	Conc
C	6.583	22.42	59.791	78.602	110.6	92.712	Conc
D	25.854	63.04	135.543	<0.000	4.388	<0.000	Conc
E	114.097	103.996	136.719	<0.000	<0.000	<0.000	Conc
F	209.394	260.584	275.487	<0.000	<0.000	<0.000	Conc
G	404.492	496.247	640.313	<0.000	<0.000	<0.000	Conc
H	882.444	840.732	>1050.000	<0.000	<0.000	<0.000	Conc

Conc x Dil

	7	8	9	10	11	12	
A				200.534	300.061	246.043	Conc x Dil
B				407.793	416.244	460.167	Conc x Dil
C				393.011	553.002	463.559	Conc x Dil
D				<0.000	43.881	<0.000	Conc x Dil
E				<0.000	<0.000	<0.000	Conc x Dil
F				<0.000	<0.000	<0.000	Conc x Dil
G				<0.000	<0.000	<0.000	Conc x Dil
H				<0.000	<0.000	<0.000	Conc x Dil

Figure 4: CCL22 concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 382 pg/mL of CCL22 was detected in the positive control (human serum) and 0 pg/mL of CCL22 was detected in the negative control (goat serum).