Validation Report #029766

Validation Date: 07/18/14

Summary

Antigen	Chemokine (C-C Motif) Ligand 2 (CCL2)
Catalog number	ABIN365052
Supplier	Cusabio
Supplier catalog number	csb-e04655h
Lot number	Z02184065
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029766
Positive Control	Human serum - expression is ~16 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 2 (CCL2)
- Catalog number: ABIN365052
- Supplier: Cusabio
- Supplier catalog number: csb-e04655h
- Lot number: Z02184065

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 using Excel. A line of best fit through the points on the graph was used to generate the equation $x = (y-0.1865) / 0.0019$.
15. The equation was used to calculate CCL2 concentrations of the samples based on their average OD values.

Experimental Notes

At high concentrations the readout of the assay is non-linear.

Figures

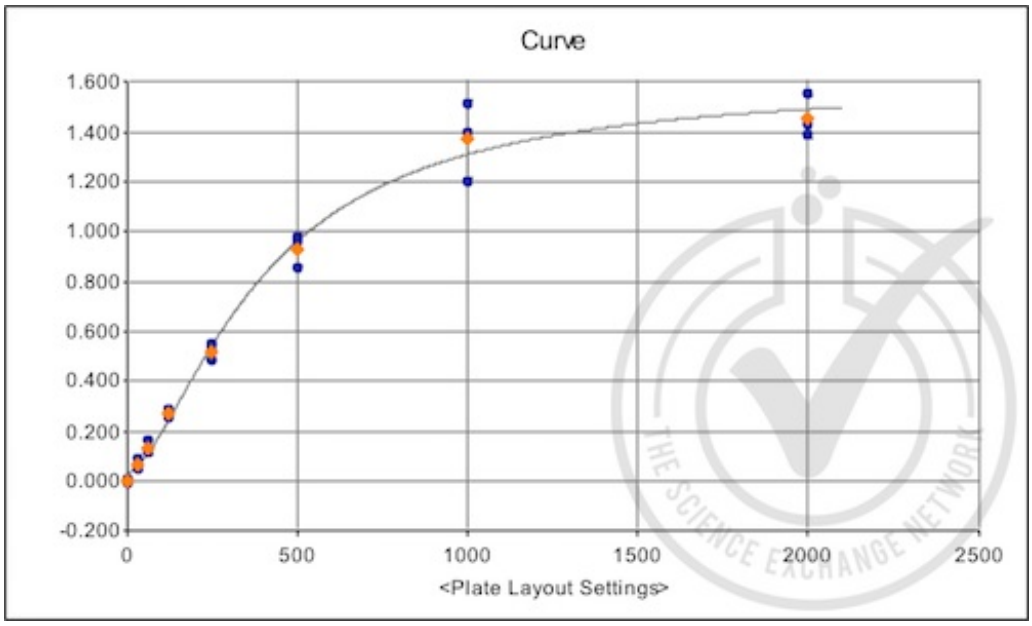

Figure 1: Human CCL2 full standard curve graph.

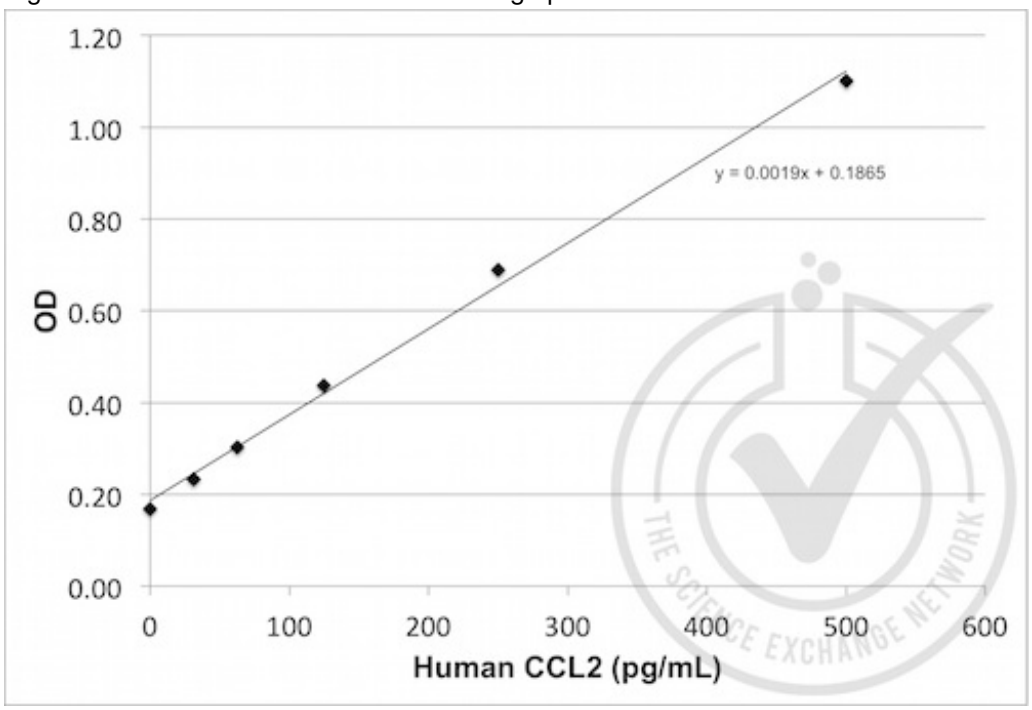

Figure 2: Human CCL2 partial standard curve graph (linear range).

	1	2	3	Average OD	Calculated CCL2 (pg/mL)
Standard curve 0 pg/mL (sample diluent)	0.16	0.17	0.17	0.17	-10.09
Standard curve 31 pg/mL	0.22	0.22	0.26	0.23	24.65
Standard curve 63 pg/mL	0.28	0.34	0.29	0.30	61.49
Standard curve 125 pg/mL	0.42	0.44	0.45	0.44	131.84
Standard curve 250 pg/mL	0.65	0.70	0.72	0.69	264.82
Standard curve 500 pg/mL	1.03	1.13	1.14	1.10	481.14
Standard curve 1000 pg/mL	1.37	1.57	1.68	1.54	712.37
Standard curve 2000 pg/mL	1.56	1.60	1.72	1.63	757.46
Positive control human serum	0.31	0.31	0.30	0.31	63.77
Positive control human serum 1:2	0.21	0.20	0.22	0.21	10.61
Positive control human serum 1:5	0.15	0.17	0.16	0.16	-14.12
Positive control human serum 1:10	0.14	0.15	0.16	0.15	-20.26
Negative control goat serum	0.08	0.08	0.08	0.08	-55.53
Negative control goat serum 1:2	0.08	0.09	0.09	0.09	-53.25
Negative control goat serum 1:5	0.15	0.10	0.10	0.11	-39.21
Negative control goat serum 1:10	0.10	0.10	0.10	0.10	-47.28

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, positive (human serum) and negative (goat serum) control samples. Value for Average Reading is derived from the average of three readings (OD 450nm). An equation $x = (y-0.1865) / 0.0019$ was generated from the partial standard curve (in the linear range) and used to calculate CCL2 concentrations shown in the Table.