Validation Report #029788

Validation Date: 08/12/14

Summary

Antigen	Cutaneous lymphocyte-associated antigen (CLA)
Catalog number	ABIN365957
Supplier	Cusabio
Supplier catalog number	csb-e09125h
Lot number	X19184064
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029788
Positive Control	Human serum - expression is ~650 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: Cutaneous lymphocyte-associated antigen (CLA)
- Catalog number: ABIN365957
- Supplier: Cusabio
- Supplier catalog number: csb-e09125h
- Lot number: X19184064

Controls

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

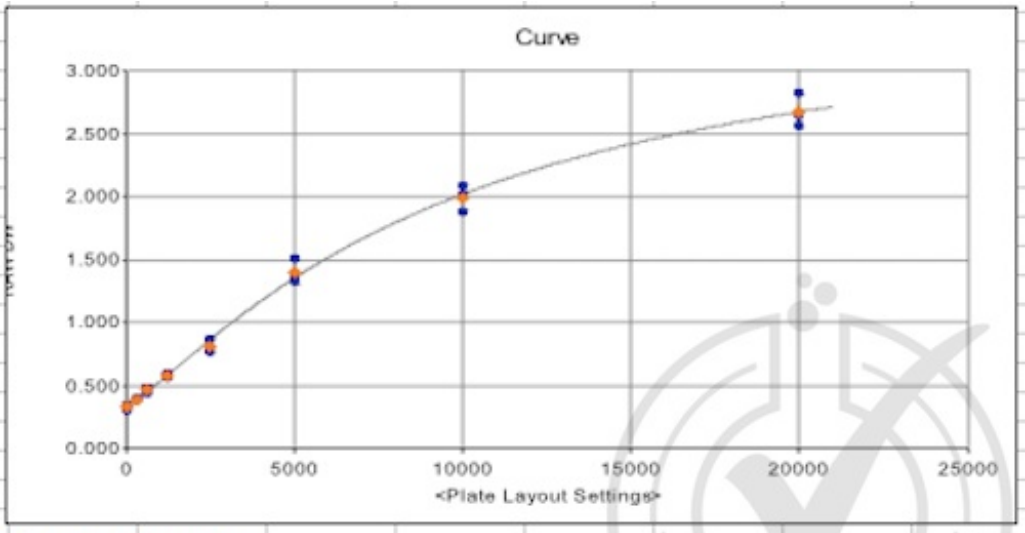
Protocol

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 CLA concentrations of the samples based on their average OD values.

Experimental Notes

Well B1 was excluded from this dataset due to possible contamination. 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	0.341	1.23	9.59E+03	3.62	0.999

Figure 1: CLA 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	SPL1:2	SPL1:2	SPL1:2	Well ID
		312.5	312.5	2	2	2	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	625	625	625	5	5	5	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	1250	1250	1250	10	10	10	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5	STD5	STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	2500	2500	2500	20	20	20	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	5000	5000	5000	40	40	40	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	10000	10000	10000	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	20000	20000	20000	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. Note that well B1 was excluded from this dataset due to possible contamination.

RAW DW

	7	8	9	10	11	12	
A	0.326	0.349	0.312	0.989	1.032	0.993	RAW DW
B		0.403	0.39	0.729	0.688	0.716	RAW DW
C	0.491	0.486	0.439	0.441	0.437	0.374	RAW DW
D	0.591	0.594	0.572	0.267	0.269	0.278	RAW DW
E	0.806	0.876	0.772	0.146	0.152	0.148	RAW DW
F	1.517	1.332	1.377	0.096	0.092	0.096	RAW DW
G	1.885	2.085	2.018	0.07	0.085	0.097	RAW DW
H	2.65	2.833	2.57	0.025	0.024	0.038	RAW DW

Figure 3: Raw OD readings of standards and controls. Note that well B1 was excluded from this dataset due to possible contamination.

Conc

	7	8	9	10	11	12	
A	<0.000	73.123	<0.000	3076.408	3284.565	3095.618	Conc
B		389.595	320.865	1880.157	1697.833	1822.256	Conc
C	815.766	792.614	569.938	579.649	560.201	231.943	Conc
D	1267.481	1280.832	1182.759	<0.000	<0.000	<0.000	Conc
E	2225.716	2545.285	2072.498	<0.000	<0.000	<0.000	Conc
F	5981.863	4862.582	5122.738	<0.000	<0.000	<0.000	Conc
G	8718.388	10627.61	9945.012	<0.000	<0.000	<0.000	Conc
H	19351.02	>21000.000	17636.95	<0.000	<0.000	<0.000	Conc

Conc x Dil

	7	8	9	10	11	12	
A				3076.408	3284.565	3095.618	Conc x Dil
B				3760.314	3395.665	3644.513	Conc x Dil
C				2898.244	2801.004	1159.713	Conc x Dil
D				<0.000	<0.000	<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: CLA concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 3013 pg/mL of CLA was detected in the positive control (human serum) and 0 pg/mL of CLA was detected in the negative control (goat serum).