Validation Report #029790

Validation Date: 08/12/14

Summary

Antigen	Interleukin 6 Signal Transducer (Gp130, Oncostatin M Receptor) (IL6ST)
Catalog number	ABIN366527
Supplier	Cusabio
Supplier catalog number	csb-e04571h
Lot number	Q03184076
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029790
Positive Control	Human serum - expression is ~310 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: Interleukin 6 Signal Transducer (Gp130, Oncostatin M Receptor) (IL6ST)
- Catalog number: ABIN366527
- Supplier: Cusabio
- Supplier catalog number: csb-e04571h
- Lot number: Q03184076

Controls

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

Protocol

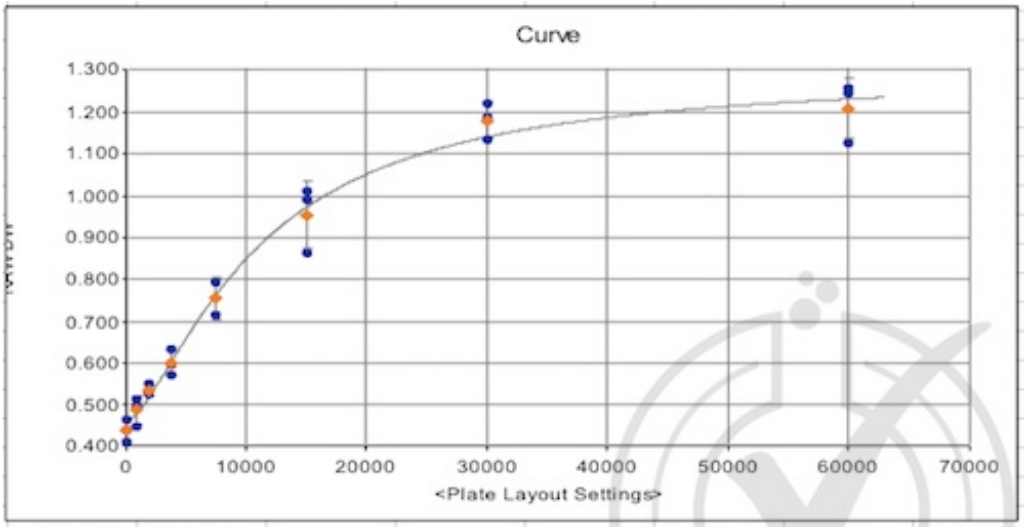
1. All reagents were brought up to room temperature for 30 minutes prior to use. The 1x Wash Buffer was prepared by adding 20 mL of 25x Wash Buffer Concentrate to 480 mL of distilled/deionized water and mixing thoroughly.
2. The vial of Standard was reconstituted with 1 mL of Sample Diluent, mixed, and 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 IL6ST concentrations of the samples based on their average OD values.

Experimental Notes

Well E2 was excluded as an outlier.

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	0.458	1.48	1.09E+04	1.29	0.995

Figure 1: IL6ST 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	10	10	10	Conc/Dil
				Human serum	Human serum	Human serum	Name
B	STD2	STD2	STD2	SPL1:2	SPL1:2	SPL1:2	Well ID
	937.5	937.5	937.5	20	20	20	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	1875	1875	1875	40	40	40	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	3750	3750	3750	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5		STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	7500		7500	100	100	100	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	15000	15000	15000	200	200	200	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	30000	30000	30000	500	500	500	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	60000	60000	60000	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 E2 was excluded as an outlier.

RAW DW

	1	2	3	4	5	6	
A	0.41	0.438	0.464	0.758	0.774	0.756	RAW DW
B	0.447	0.498	0.514	0.622	0.634	0.545	RAW DW
C	0.525	0.526	0.55	0.46	0.429	0.425	RAW DW
D	0.596	0.572	0.634	0.403	0.421	0.395	RAW DW
E	0.716		0.791	0.835	0.743	0.738	RAW DW
F	0.862	0.99	1.01	0.225	0.214	0.208	RAW DW
G	1.133	1.221	1.188	0.183	0.177	0.188	RAW DW
H	1.127	1.244	1.255	0.082	0.081	0.07	RAW DW

Figure 3: Raw OD readings of standards and controls. Note that well E2 was excluded as an outlier.

Conc

	1	2	3	4	5	6	
A	<0.000	<0.000	416.202	7407.411	7829.328	7355.655	Conc
B	<0.000	1470.565	1865.748	4237.877	4498.047	2574.547	Conc
C	2123.534	2146.56	2684.848	217.171	<0.000	<0.000	Conc
D	3678.758	3163.228	4498.047	<0.000	<0.000	<0.000	Conc
E	6360.866		8294.175	9591.022	7024.214	6898.93	Conc
F	10467.72	15973.7	17146.7	<0.000	<0.000	<0.000	Conc
G	28839.15	53700.24	40396.31	<0.000	<0.000	<0.000	Conc
H	27963.22	>63000.000	>63000.000	<0.000	<0.000	<0.000	Conc

Conc x Dil

	1	2	3	4	5	6	
A				74074.11	78293.28	73556.55	Conc x Dil
B				84757.55	89960.94	51490.94	Conc x Dil
C				8686.832	<0.000	<0.000	Conc x Dil
D				<0.000	<0.000	<0.000	Conc x Dil
E				959102.2	702421.4	689893	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: IL6ST concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 75356 pg/mL (75 ng/mL) of IL6ST was detected in the positive control (human serum) and 0 pg/mL of IL6ST 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 IL6ST level in human serum.