Validation Report #029784

Validation Date: 08/11/14

Summary

Antigen	Mannma Binding Protein/mannan Binding Lectin (MBP/MBL)
Catalog number	ABIN367216
Supplier	Cusabio
Supplier catalog number	csb-e09717h
Lot number	Y25184072
Method validated	Enzyme-linked immunosorbent assay
Laboratory	CGIBD Advanced Analytics Core
Validation number	029784
Positive Control	Human serum - expression is ~50 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: Mannma Binding Protein/mannan Binding Lectin (MBP/MBL)
- Catalog number: ABIN367216
- Supplier: Cusabio
- Supplier catalog number: csb-e09717h
- Lot number: Y25184072

Controls

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

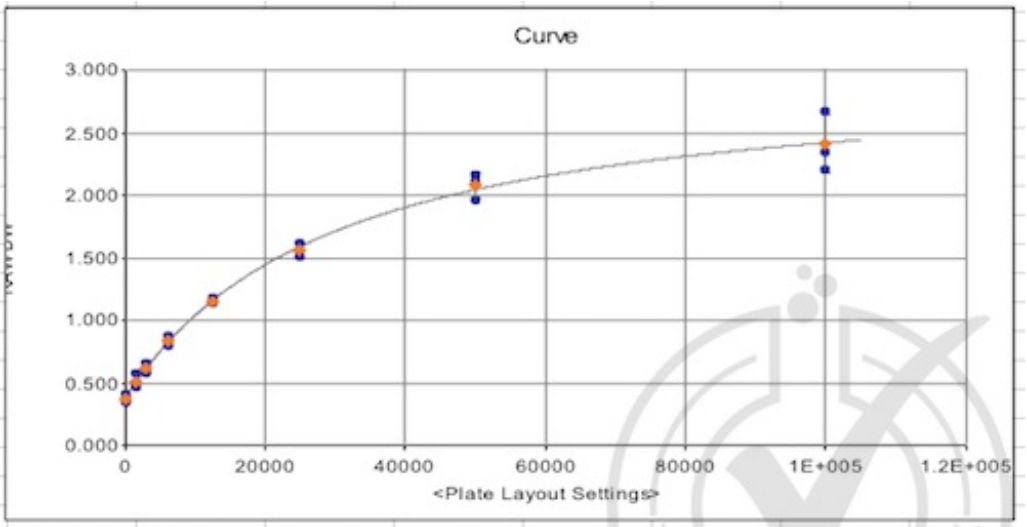
Protocol

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

Experimental Notes

The 100x HRP-avidin provided contained less than 60 µL. 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	0.383	1.03	2.86E+04	2.99	0.999

Figure 1: MBP 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	50	50	50	Conc/Dil
				Human serum	Human serum	Human serum	Name
B	STD2	STD2	STD2	SPL1:2	SPL1:2	SPL1:2	Well ID
	1562.5	1562.5	1562.5	100	100	100	Conc/Dil
				Human serum	Human serum	Human serum	Name
C	STD3	STD3	STD3	SPL1:3	SPL1:3	SPL1:3	Well ID
	3125	3125	3125	200	200	200	Conc/Dil
				Human serum	Human serum	Human serum	Name
D	STD4	STD4	STD4	SPL1:4	SPL1:4	SPL1:4	Well ID
	6250	6250	6250	500	500	500	Conc/Dil
				Human serum	Human serum	Human serum	Name
E	STD5	STD5	STD5	SPL1:5	SPL1:5	SPL1:5	Well ID
	12500	12500	12500	1000	1000	1000	Conc/Dil
				Human serum	Human serum	Human serum	Name
F	STD6	STD6	STD6	SPL1:6	SPL1:6	SPL1:6	Well ID
	25000	25000	25000	2000	2000	2000	Conc/Dil
				Human serum	Human serum	Human serum	Name
G	STD7	STD7	STD7	SPL1:7	SPL1:7	SPL1:7	Well ID
	50000	50000	50000	5000	5000	5000	Conc/Dil
				Human serum	Human serum	Human serum	Name
H	STD8	STD8	STD8	SPL2	SPL2	SPL2	Well ID
	1.00E+05	1.00E+05	1.00E+05	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	0.358	0.356	0.415	2.199	2.152	2.066	RAW DW
B	0.475	0.497	0.58	2.341	2.265	2.413	RAW DW
C	0.582	0.612	0.663	1.16	1.124	1.174	RAW DW
D	0.805	0.844	0.89	0.751	0.732	0.755	RAW DW
E	1.185	1.142	1.156	0.48	0.494	0.465	RAW DW
F	1.516	1.561	1.626	0.316	0.318	0.3	RAW DW
G	2.121	1.964	2.168	0.185	0.188	0.178	RAW DW
H	2.352	2.215	2.672	0.05	0.053	0.045	RAW DW

Figure 3: Raw OD readings of standards and controls.

Conc

	1	2	3	4	5	6	
A	<0.000	<0.000	398.863	64208.65	59179.21	51274.97	Conc
B	1145.308	1423.618	2506.451	83724.45	72347.48	97206.87	Conc
C	2533.263	2939.912	3651.504	12447.95	11663.73	12760.92	Conc
D	5786.171	6417.095	7188.974	4945.768	4658.726	5006.757	Conc
E	13010.08	12052.21	12359.37	1208.286	1385.477	1019.8	Conc
F	22167.26	23727.23	26156.35	<0.000	<0.000	<0.000	Conc
G	56153.62	43583.4	60828.14	<0.000	<0.000	<0.000	Conc
H	85591.02	66056.98	>105000.000	<0.000	<0.000	<0.000	Conc

Conc x Dil

	1	2	3	4	5	6	
A				3210433	2958960	2563748	Conc x Dil
B				8372445	7234748	9720687	Conc x Dil
C				2489591	2332746	2552185	Conc x Dil
D				2472884	2329363	2503379	Conc x Dil
E				1208286	1385477	1019800	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: MBP concentrations calculated from standard curve formula. Upper panel = uncorrected for dilution; lower panel = corrected for dilution. On average, 3490315 pg/mL of MBP was detected in the positive control (human serum) and 0 pg/mL of MBP was detected in the negative control (goat serum).