Last Edited 7-10-18

This protocol can be used for B -1-6 and B 1-3 Glucan ELISA. There are two things one needs to remember; First, prepare standard curve with the carbohydrate you wish to test, either B 1-6 or B-1-3 Glucan , second, make sure you use the appropriate first antibody for the assay and standard curve.

Note**: PBS + 0.1% BSA + 0.001 % Tween-20** is used for all antibody dilutions and **100µl/well** is added to the wells in **ALL** of the antibodies steps. Use **300 ul/well** for all of the washes.

In a WHITE 96 well plate (NUNC # 436110)) set up a standard curve with the antigen (glucan etc) in PBS. Do half dilutions starting with 1000ng/ml, 500, 250, 125 etc. and use 3 wells per standard.

ELISA Plate Template

Std curve Controls Blank Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	500	250	125	62	31	15	8	1st	2nd	perOx	Sub
B	1000	500	250	125	62	31	15	8	1st	2nd	perOx	Sub
C	1000	500	250	125	62	31	15	8	1st	2nd	perOx	Sub
D	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	blank
E	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	blank
F	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	blank
G	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	blank
H	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	blank

1- On remaining wells load samples in triplicates, (If you have plenty of sample material you can do four wells per sample).

2- Incubate **overnight at 4°C.**

3- Decant fluid from wells and block with PBS containing 2% BSA + 0.02% Tween-20 , incubate for 45 min at RT. (Tween -20 makes a huge difference so don't forget to add it to the blocking buffer)

4- Decant blocking solution and add 1st antibody, Mab anti- B-1-3 at **1:5000** dilution

5- Incubate at RT for 1.5hr.

6- Wash plate 3 times in plate washer, decant leftover fluid.

7- Add goat anti-mouse IgG-HRP at **1:1000 dilution**. (Pierce) Incubate at RT for 1.5 hr.

8- After incubation, wash again and decant fluid.

9- Prepare Luminol substrate in a 15 ml conical tube by mixing 5ml of diluent and 5 ml of Luminol, (Pierce Cat # 37070) add 100ul to each well and gently shake for 3 min. Make sure there are no bubbles in the wells.

10- Incubate for 30 min at RT in the dark, Read plate with the luminescence software or using the B 1-6 ELISA program already in the program directory.

11- The software will calculate and plot the standard curve and will calculate the concentrations for you. Save your results in your own folder.

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