



---

# ELISA TITRATION

**Created/updated by: Samantha Balachandran Date: April 15, 2013**

Bowdish Lab, McMaster University  
Hamilton, ON, Canada

[www.bowdish.ca](http://www.bowdish.ca)

## BACKGROUND

- In order to obtain optimal quantification analyses from an ELISA, a robust standard curve needs to be set. When generating the optimal standard curve to fit your needs, some time might have to be spent with varying concentration ranges of the standard, the primary antibody and the detection antibody. Thus, this protocol outlines the manipulation of these three variables in achieving the optimal standard curve for your ELISA.

## NOTES

- This protocol requires two- three days, just as an ELISA. Ensure that the wash and assay solutions are prepared before hand

## EQUIPMENT

- The plate washer and the spectrophotometer are needed

## PROTOCOL

- Coat 96-well plate (maxi sorb tubes) with capture antibody (diluted in coating buffer) and let it incubate overnight
- Wash plate with ~300 ul wash buffer (0.05% tween in PBS) once
- Block the plate for 1 hour in room temperature or overnight at 4 degrees with assay diluent (10% FBS in PBS)
- Wash plate wash buffer 3x
- Place 100 ul/well of standards (diluted in assay diluent) and allow to incubate for 2h in room temperature or overnight at 4 degrees
- Wash the plate with wash buffer 3x – 5x
- Place 100 ul/well of detection antibody (diluted in assay diluent) and incubate for 1h at room temperature
- Wash plate with wash buffer 3x – 5x
- Place 100 ul/well of Strep Avidin HRP (diluted in assay diluent) and incubate for 30 minutes at room temperature

- Wash plate with wash buffer 5x – 7x
- Add 100 ul/well of substrate solution and incubate for ~ 15 minutes at room temperature (until a gradient is visible in the standards)
- Add 50 ul/well of stop solution (2M H<sub>2</sub>SO<sub>4</sub>)
- Read plate at 450 nm

PLEASE NOTE THAT THESE RANGES ARE MEANT FOR sCD14 AND CAN BE CHANGED AS NEEDED

TITRATION PLATE MAP

- capture antibody recommended range: 1 - 4 ug/ml
- standard recommended range: 20 ng/ul – 200 ng/ul
- detection antibody recommended range: 0.5 - 1 ug/ml
- streptavidin HRP 1:500

	1	2	3	4	5	6	7	8	9	10	11	12	Capture Ab	Strep:HRP
A	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	1 ug/ml	1:500
B	Standard conc. (ng/ml)				Standard conc. (ng/ml)				Standard conc. (ng/ml)					1:250
C	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	2 ug/ml	1:500
D	Standard conc. (ng/ml)				Standard conc. (ng/ml)				Standard conc. (ng/ml)					1:250
E	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	3 ug/ml	1:500
F	Standard conc. (ng/ml)				Standard conc. (ng/ml)				Standard conc. (ng/ml)					1:250
G	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	4 ug/ml	1:500
H	Standard conc. (ng/ml)				Standard conc. (ng/ml)				Standard conc. (ng/ml)					1:250
<b>De tab</b>	0.2 ug/ml				0.5 ug/ml				1 ug/ml					