HUMAN CRP ELISA

Created by: Mohammad Malik **Date:** April 24th, 2018
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

BACKGROUND

- For detection of C-Reactive Protein (CRP) in human samples.
- Can be done with human plasma

MATERIALS

- Equipment
  - P1000, P100, P10 pipettors and tips
  - Multipipettor
  - 1.5 mL Eppendorf tubes
  - NUNC ELISA well plate inserts (flat-bottomed)
  - Reagent reservoirs
  - Plate washer
  - i3 Plate Reader
  - ELISA plate seals
- Reagents
  - CRP Capture Antibody (abcam8279, stock concentration = 2 mg/mL)
  - CRP Detection Antibody (abcam24462, stock concentration = 0.8 mg/mL)
  - Coating Buffer (1.5g Anhydrous Na_2CO_3 + 2.93g Anhydrous $NaHCO_3$ + 1L Distilled Water, pH 9.6)
  - Recombinant human CRP (stored in -80°C freezer)
  - Blocking Buffer (PBS + 1% w/v BSA, filter-sterilized)
  - Wash Buffer (PBS + 0.05% v/v Tween-20)
  - TMB Substrate
  - 2N H_2SO_4

PROCEDURE

- Coat desired number of wells with 100uL/well of capture antibody diluted to a concentration of 1ug/mL in coating buffer.
- Seal plate and store at 4C O/N.
- Dump contents and fill wells with 300uL blocking buffer. Seal plate and incubate for 30mins-1h at RT on a shaker.
  - At this point you can also incubate overnight.
- Dump contents and tap plate dry onto a few napkins.
- Load 100uL/well in duplicate for each standard and sample.

- Standard curve (made using recombinant human CRP) in ng/mL: 750, 500, 250, 125, 31.25, 7.813, 1.953, 0; diluted in blocking buffer. Follow the table below to ensure accurate dilution of standards:

	750ng/mL	**500ng/mL**	**250ng/mL**	**125 ng/mL**	**31.25 ng/mL**	**7.813ng/mL**	**1.953ng/mL**	**0ng/mL**
Buffer	595.5 uL	150 uL	225 uL	225 uL	375 uL	375 uL	375 uL	225 uL
Stock (0.1mg/mL)	4.5 uL							
750ng/mL		300 uL						
500ng/mL			225 uL					
250ng/mL				225 uL				
125 ng/mL					125 uL			
31.25 ng/mL						125 uL		
7.813ng/mL							125 uL	
Total Volume	600 uL	450 uL	450 uL	450 uL	500 uL	500 uL	500 uL	225 uL
Well Volume	200 uL	200 uL	200 uL	200 uL	200 uL	200 uL	200 uL	200 uL
Dilution Volume	300 uL	225 uL	225 uL	225 uL	125 uL	125 uL	0 uL	0 uL
Extra Volume	100 uL	25 uL	25 uL	25 uL	175 uL	175 uL	300 uL	25 uL

    - Plasma samples are recommended to be diluted Between 1/200 and 1/1000 in blocking buffer.
- Seal plate and incubate for 2 hours at RT on a shaker.
- Wash plate (3X) with wash buffer using plate washer.
- Add 100uL/well of detection antibody at 1ug/mL in blocking buffer.
- Seal plate and incubate for 1 hour at RT on shaker.
    - While the plate is incubating, take the TMB substrate out of the fridge so that it can come to room temperature for the next step.
- Wash plate (5X) with wash buffer using plate washer.
- Add 100uL/well of TMB substrate and give the plate a gentle shake. Develop plate until sufficient colour change is seen (nice separation in the standard curve, and most samples are within the standard curve range).
    - Have 2N H_2SO_4 ready to stop the reaction as the plate may develop very quickly.
- Add 50uL of 2N H_2SO_4 to stop the reaction and shake the plate gently.
- Read absorbance at 450nm using plate reader.

APPENDIX

Plasma is recommended for this assay. Both serum and plasma were tested and serum did not respond to multiple dilutions (see Figure 1). A dilution between 1/200 and 1/1000 is recommended because dilutions of 1/200, 1/500 and 1/1000 yielded similar concentrations after adjusting for the dilution factor (see Figure 2).

Figure 1

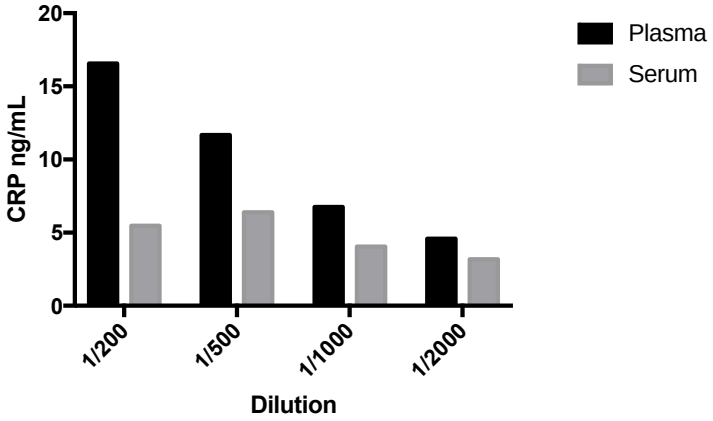
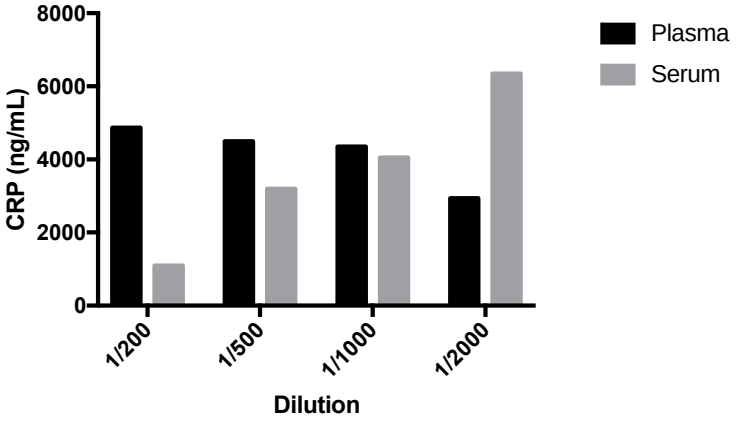


Figure 2



Standard Curve: