MOUSE C-REACTIVE PROTEIN (CRP) ELISA
Life Diagnostics, Inc., Catalog Number: CRP-1

INTRODUCTION

CRP is an acute phase protein in that is elevated in serum due injury, infection or disease.[1,2] In studies at Life Diagnostics, Inc. we found that serum levels of CRP were 2.68 ± 0.17 μg/ml (mean ± SD, n=5) in normal BALB/c mice. Twenty-four hours after LPS injection, levels increased to 10.34 ± 4.61 μg/ml (mean ± SD, n=5). Measurement of CRP provides a convenient marker of inflammation and disease in mice.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified mouse CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse CRP antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in CRP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If CRP is present a blue color develops. Color development is stopped by the addition of Stop solution; changing the color to yellow, and absorbance is measured at 450 nm. The concentration of CRP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit*:*

- CRP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- CRP stock (lyophilized). **Store at -20°C**
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: CSD50-1, 50 ml
- TMB: TMB11-1, 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The CRP stock should be stored at or below -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Reconstitute the lyophilized mouse CRP reference stock as indicated on the vial label. Unused reconstituted stock should be aliquoted and stored frozen at or below -20°C.
2. Label 7 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
3. In the tube labeled 100 ng/ml prepare the 100 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 μl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Prepare the 50 ng/ml standard by mixing 250 μl of the 100 ng/ml standard with 250 μl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found that CRP is present in mouse serum at concentrations of 2.5 - 20 μg/ml. We suggest that serum samples be diluted 200-fold for use in this assay. Do not use dilutions below 50-fold (i.e., 25-fold) because other serum components interfere with CRP measurement. A 200-fold sample dilution can be prepared as follows.

1. Dispense 398 μl of diluent into separate tubes.
2. Pipette and mix 2.0 μl of the serum sample into the tube containing 398 μl of diluent. This provides a 200-fold diluted sample.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.

Life Diagnostics, Inc., PO Box 5205 West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

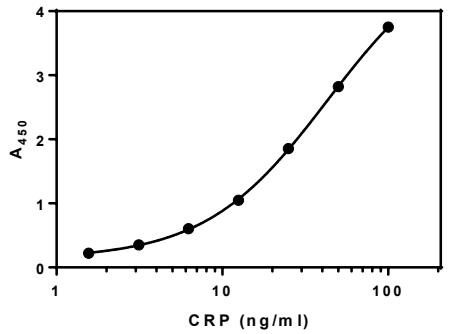
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CRP (ng/ml)	Absorbance (450 nm)
100	3.750
50	2.822
25	1.854
12.5	1.047
6.25	0.604
3.13	0.352
1.56	0.221



ASSAY CHARACTERISTICS

Typical results obtained with BALB/c mouse serum samples are shown in the table below. Samples 836-840 were from control mice. Samples 856-860 were collected from mice 24 hours after LPS injection. Samples were diluted as indicated and concentrations (ng/ml) determined from a standard curve. Serum concentrations (SC, µg/ml) were calculated by multiplying the concentrations of the samples by their respective dilution factors. Average values, standard deviation (SD) and coefficients of variation (CV) were then calculated.

Sample	Dilution	A_{450}	ng/ml	SC (ug/ml)	Average (ug/ml)	SD (ug/ml)	CV
836	50	2.676	49.5	2.47	2.44	0.06	2.6
	100	1.701	24.7	2.47			
	200	0.994	12.3	2.46			
	400	0.553	5.9	2.34			
837	50	2.908	57.9	2.90	2.85	0.12	4.2
	100	1.926	29.4	2.94			
	200	1.127	14.4	2.88			
	400	0.613	6.7	2.67			
838	50	2.833	55.0	2.75	2.75	0.12	4.3
	100	1.900	28.9	2.89			
	200	1.092	13.8	2.77			
	400	0.599	6.5	2.59			
839	50	2.879	56.8	2.84	2.80	0.12	4.2
	100	1.919	29.3	2.93			
	200	1.096	13.9	2.78			
	400	0.608	6.6	2.65			
840	50	2.730	51.3	2.56	2.58	0.12	4.5
	100	1.822	27.2	2.72			
	200	1.040	13.0	2.60			
	400	0.570	6.1	2.43			
856	200	2.713	50.7	10.14	9.87	0.22	2.2
	400	1.697	24.6	9.86			
	800	0.996	12.3	9.86			
	1600	0.564	6.0	9.61			
857	200	3.482	93.2	18.63	18.19	0.37	2.1
	400	2.558	45.7	18.28			
	800	1.596	22.7	18.14			
	1600	0.915	11.1	17.73			
858	200	2.024	31.6	6.32	6.19	0.15	2.5
	400	1.179	15.3	6.10			
	800	0.672	7.5	6.01			
	1600	0.411	3.9	6.31			
859	200	2.547	45.4	9.07	8.07	0.69	8.5
	400	1.416	19.3	7.74			
	800	0.837	9.9	7.92			
	1600	0.469	4.7	7.54			
860	200	2.768	52.6	10.53	9.41	1.33	14.1
	400	1.782	26.4	10.54			
	800	0.897	10.8	8.64			
	1600	0.487	5.0	7.93			

REFERENCES

1. Schreiber G. et al. The acute phase response in the rodent. Ann N Y Acad. Sci. 557:61-85 (1989)
2. Patterson LT and Higginbotham ED. Mouse C-Reactive Protein and Endotoxin-Induced Resistance. J Bacteriology. 90: 1520-1524 (1965)

Rev 09152020

For technical assistance please email us at techsupport@lifediagnostics.com