

# RABBIT C-REACTIVE PROTEIN (CRP) ELISA

## Life Diagnostics, Inc., Catalog Number: CRP-10-N

### INTRODUCTION

CRP is an acute phase protein in rabbits that is elevated in serum because of injury, infection, or disease; CRP levels can increase several hundred-fold.<sup>1-4</sup> Measurement of CRP therefore provides a convenient marker of inflammation and disease in rabbits. Life Diagnostics rabbit CRP ELISA kits were used to identify CRP as a biomarker of acute inflammation in vaccine toxicity studies.<sup>4</sup>

### PRINCIPLE OF THE ASSAY

The assay uses affinity purified rabbit CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rabbit CRP antibodies for detection. The antibodies were generated in chickens using pure rabbit CRP as immunogen and affinity purified on rabbit CRP agarose. In the assay, 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. 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. 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. **Store at -20°C**
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: YD50-1, 2 x 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 lyophilized standards must be stored at or below -20°C when received. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase if stored as described.

### 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

The rabbit CRP stock is comprised of lyophilized rabbit serum of known CRP concentration. The CRP content was determined by reference to purified rabbit CRP prepared at Life Diagnostics, Inc.

1. Reconstitute the stock as detailed on the vial label.
2. Label eight polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume CRP stock and mix. This provides the 50 ng/ml standard.
4. Dispense 250 µl of 1x diluent into the remaining tubes.
5. Pipette 250 µl of the 50 ng/ml CRP standard into the tube labeled 25 ng/ml and mix. This provides the 25 ng/ml CRP standard.
6. Similarly prepare the remaining standards by serial dilution.

Please Note: The unused reconstituted stock should be aliquoted and stored frozen at or below -20°C (within 1 hour of reconstitution) if future use is intended.

### SAMPLE PREPARATION

CRP is present in rabbit serum at concentrations ranging from less than 1 µg/ml to >100 µg/ml. We found that dilutions of both 100-fold and 5000-fold worked well for normal and acute-phase samples, respectively. Optimum dilutions should be determined empirically. To avoid matrix effects, serum must be diluted at least 20-fold.

### 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  $\mu$ l 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.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100  $\mu$ l of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100  $\mu$ l 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.

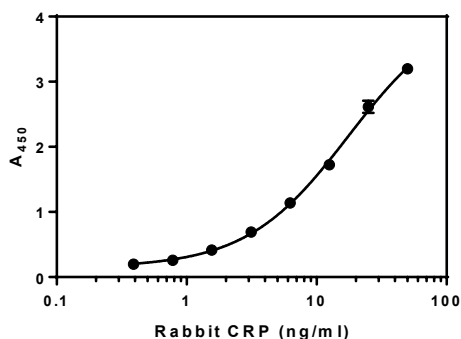
### CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus log<sub>10</sub> of the concentration.
2. Fit the standard curve to a four-parameter logistic equation ( $x = \log_{10}$  concentration and derive the concentration of the samples (derive concentrations from the anti-log).
3. Multiply the derived concentration by the dilution factor to determine the concentration in the serum 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

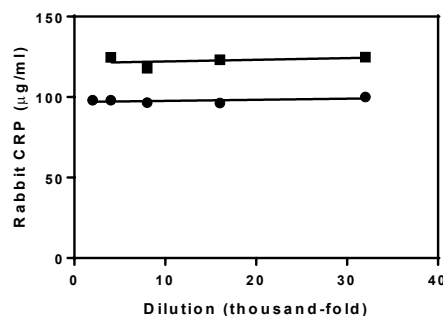
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CRP (ng/ml)	Absorbance (450 nm)
50	3.197
25	2.615
12.5	1.726
6.25	1.139
3.13	0.691
1.56	0.416
0.78	0.259
0.39	0.198



### PERFORMANCE

**Parallelism.** To assess parallelism of the assay, serum samples with CRP concentrations of 96 and 125  $\mu$ g/ml were serially diluted to produce values within the dynamic range of the assay. Although not shown, linearity was also obtained for samples with CRP <1  $\mu$ g/ml when tested at dilutions ranging from 20- to 1000-fold.



### Comparison with kit CRP-10.

ELISA kit CRP-10-N uses chicken anti-rabbit CRP antibodies that were generated by injecting chickens with pure rabbit CRP. They were affinity purified on rabbit CRP agarose. The standard used in kit CRP-10-N was calibrated to pure rabbit CRP. The concentration of which was determined by absorbance at 280 nm, assuming an Abs 0.1% of 2.077, derived from the amino acid sequence of rabbit CRP.

ELISA kit CRP-10 uses goat anti-human CRP antibodies that cross-react with rabbit CRP. The standard was calibrated to pure rabbit CRP, the concentration of which was determined by Bradford assay using BSA as reference protein standard.

Samples with a CRP concentration of 10  $\mu$ g/ml, as measured with kit CRP-10, had a concentration of 4.29  $\mu$ g/ml when measured with kit CRP-10-N. Differences are attributable to the different methods used to determine CRP concentration.

### REFERENCES

1. Qian Z. et. al. Targeting vascular injury using hantavirus-pseudotyped lentiviral vectors. *Molecular Therapy* 13:694-704 (2006)
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4. Destexhe E. et. al. Evaluation of C-reactive protein as an inflammatory biomarker in rabbits for vaccine nonclinical safety studies. *J. Pharmacological and Toxicological Methods.* 68:367-373 (2013)

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