



## CRP ELISA

Catalog Number EA-0303

(For Research Use Only)

### Introduction

C-reactive protein (CRP) was named for its ability to bind to and precipitate the C-polysaccharide of pneumococcus (1-2). CRP is synthesized in the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl (2-3). Although its physiological roles are numerous and varied, CRP appears to function in host defense (1). CRP is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases, including infections by gram-positive and gram-negative organisms, acute phase of rheumatoid arthritis, abdominal abscesses, and inflammation of the bile duct (4). CRP may also be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases, burned patients and after surgical trauma (4-6). Several prospective studies have demonstrated a direct correlation between acute myocardial infarction (MI) rise in CRP, postinfarction adverse events (7-8) and subsequent infarct size (9). Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes (10). CRP levels rise in serum or plasma within 24 to 48 hours following acute tissue damage, reach a peak during the acute stage (approximately 1000x constitutive level) and decrease with the resolution of inflammation or trauma (1, 11-12). The concentration increase of CRP in human serum or plasma may last for several days before decreasing to normal levels (11-13).

### Principle of the assay

The CRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse monoclonal antibody against distinct determinants on CRP for immobilization on the microtiter wells and a goat anti-CRP antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in CRP being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of CRP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

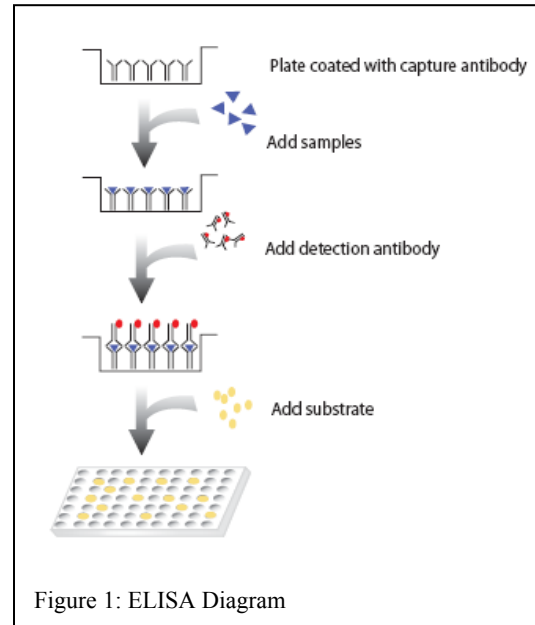


Figure 1: ELISA Diagram

### Materials provided with the kit

- Mouse monoclonal anti-CRP antibody-coated plate with 96 wells.
- Reference Standard Set (1.0 ml/vial) Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/l CRP in phosphate buffer-BSA solution with preservatives; lyophilized.
- hsCRP Sample Diluent (50 ml/vial) Contains phosphate buffer-BSA solution with preservatives.
- Enzyme Conjugate Reagent, 12 ml
- TMB Reagent (one step), 11 ml
- Stop Solution (1N HCl), 11 ml.

## Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Warning and precautions

1. Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (14) or other appropriate national biohazard safety guidelines or regulations (15-17).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

## Reagent preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.

Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8°C.

Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (-20°C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

## Assay procedure

1. Patient serum and control serum should be diluted 100 folds prior to use.
2. Add 10µl of CRP standards, diluted specimens, and diluted controls into appropriate wells.
3. Add 100µl of CRP Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Add 100µl TMB solution into each well. Gently mix for 5 seconds.

9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100µl of Stop Solution to each well.
11. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

## References

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**Example of standard curve**

CRP (mg/l)	Absorbance (450 nm)
0	0.073
0.005	0.358
0.010	0.624
0.025	1.305
0.050	2.093
0.100	2.962

