



C-Reactive Protein Assay

Intended Use:

C-Reactive Protein is used as an *in vitro* diagnostic marker to aid in the evaluation of the amount of injury to human body tissue. Produced mainly in the liver, C-Reactive Protein is a plasma protein whose concentration increases dramatically during the acute phase inflammatory response. Inflammation and other pathological processes such as coronary artery disease, stroke, heart attack, and others may cause CRP levels to be elevated as much as 2000 fold. CRP is also useful as a prognostic indicator for the risk of cardiovascular disease in general. CRP levels are very helpful in determining not only the degree of damage from a cardiac episode as related to the post-CRP concentration, but also to aid in assessing the risk of future serious cardiac conditions associated with inflammation and other pathology.

Principle of Procedure:

Solid phase capture sandwich ELISA assay using a microwell format.

Shelf Life:

The expiration date for the package and each component is stated on the label(s). Store all components at 2-8°C except for standard, which should be stored frozen.

Patient and Standard Dilutions:

Standards are provided pre-diluted 1:10,000. Prepare serial two fold dilutions of the CRP standard: Neat, 1:2, 1:4, 1:8 etc. with the specimen diluent provided. Patient specimens should be diluted to at least 1:10,000. Sample may be diluted initially with Phosphate buffered saline (PBS), *e.g.* 10ul specimen into 10 ml PBS, then sub dilute 1:10 with the specimen diluent provided for a final dilution of 1:10,000. Greater dilutions may be necessary in order to get them on the standard curve used (use specimen diluent for any dilutions beyond 1:10K). A mathematical correction should be applied to correct for the difference between the dilution used for standard verses specimen. For example if both are diluted the same there is no correction factor. But if specimens were diluted 1:20,000 and the standard curve used had an initial dilution of 1: 10,000 then interpolated answers would be multiplied by a factor of two.

Materials Supplied:

Anti-CRP microwell coated strip 8 x 12 with plastic frames
HRP conjugated anti-human CRP – 12 mL
CRP standard – 1 mL
TMB/peroxide substrate color developer – 12 mL
CRP Specimen Diluent (Specimen Diluent Green II)– 60 mL
Sulfuric acid termination reagent (0.5N) – 12 mL
15X Wash buffer concentrate – 60 mL

Limitations of the Procedure:

No single assay should be used as the only basis for arriving at a diagnostic conclusion. For research use only.

Dynamic Range:

0.12ug/dL to 4.8ug/dL.

Reproducibility:

C.V.% 2-6% depending upon the region of the standard curve.

Assay Procedure:

- 1: Dilute specimen 1:10,000 with specimen diluent.
- 2: Prepare serial two fold dilutions (at least 5 subsequent) of the standard to obtain a standard curve. (Standards should be run in at least duplicates.)
- 3: Add 100uL of diluted specimen or standard to each microwell.
- 4: Incubate at room temperature for one hour (22°-25° C).
- 5: Decant and wash 5 times with diluted wash buffer (dilute buffer 1:15 with reagent grade water.)
- 6: Add 100uL of the HRP conjugated Anti-human CRP to each well.
- 7: Incubate at room temperature for one hour.
- 8: Repeat washing as in step 4.
- 9: Add 100 uL of TMB/Peroxide to each well.
- 10: Incubate at room temperature for 30 minutes.
- 11: Add 100uL of 0.5N sulfuric acid to each well to terminate color development.
- 12: Read the optical density at 450nm using a standard microwell plate reader.

Typical Standard Curve:

