High Sensitivity C-Reactive Protein

ENZYME IMMUNOASSAY TEST KIT
Catalog No.10603

High Sensitivity Enzyme Immunoassay for the Quantitative Determination of C-Reactive Protein Concentration in Human Serum

Principle of the Assay

The hsCRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the two antibodies and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. The tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution and the color is measured at 450 nm.

Materials Required But Not Provided

- 1. Antibody-Coated Wells (1 plate, 96 wells)
- 2. Microtiter wells coated with mouse monoclonal anti-CRP
- 3. Reference Standard Set (1.0 mL/vial) Contains 5, 10, 25, 50 and 100 ng/mL CRP in liquid with preservatives, ready to use.
- 4. hsCRP Sample Diluent (60 mL/vial)
- 5. CRP Enzyme Conjugate Reagent (12 mL/vial)
- 6. TMB Reagent (12 mL/bottle)
- 7. Stop Solution (1 bottle, 12 mL/bottle)
- 8. Wash Buffer Concentrate (50X), 15 mL
- 9. Graph paper

Warnings and Precautions

1. CAUTION: This kit contains human material. The source material used for manufacturing the kit was tested negative for HIV-1, HBV, HCV by FDA-approved methods. However, no method can completely assure absence of infectious agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Avoid contact with the stop solution. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and Seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different expiration dates.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For in vitro diagnostic use.

Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-22°C) before use.
2. Patient serum should be diluted 100 fold prior to use. Prepare a series of small tubes (i.e., 1.5 mL microcentrifuge tubes) and mix 5 mL of serum with 500 mL (0.500 mL) Sample Diluent. DO NOT DILUTE THE STANDARDS.
3. Samples with expected CRP concentrations over 10 mg/L may be quantitated by doubling the dilution of the 10-fold diluted sample with sample diluent (1:10 of the 100-fold diluted sample). It is very important to mix completely. Rinse and mix the microtiter wells 5 times with washing buffer X3 for 30 seconds.
4. Stroke the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Dispense 100 µL of CRP Enzyme Conjugate Reagent into each well. It is very important to mix completely. Rinse and mix the microtiter wells 5 times with washing buffer X3 for 30 seconds.
6. Dispense 100 µL of Sample diluted into each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
7. Incubate at room temperature (18-22°C) for 30 minutes.
8. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and mix the microtiter wells 5 times with washing buffer X3 for 30 seconds.
9. Dispense 100 µL of TMB Reagent into each well. Thoroughly mix for 30 seconds. Then Incubate at room temperature (18-22°C) for 30 minutes.
10. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and mix the microtiter wells 5 times with washing buffer X3 for 30 seconds.
11. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
12. Dispense 100 µL TMB Reagent into each well. Gently mix for 5 seconds.
13. Incubate at room temperature (18-22°C) for 30 minutes.
14. Stop the reaction by adding 100 µL of Stop Solution to each well.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Quality Control

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

Calculation of Results

1. Calculate the mean absorbance value (OD mean) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mg/L on graph paper, with absorbance on the vertical(y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP (ng/mL) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in ng/mL. Patient samples with CRP concentrations greater than 10 mg/L (10,000 ng/mL) should be further diluted 10-fold or more after the initial 100-fold dilution (total dilution factor of 1,100 or more). Bialy’s final CRP values should be multiplied by 1,100 or more to obtain CRP results in ng/mL.

**Example of Standard Curve**

Results of a typical run with absorbivity readings at 450nm shown on the y axis against CRP concentrations shown on the x axis. NOTE: This curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>CRP (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.041</td>
</tr>
<tr>
<td>5</td>
<td>0.136</td>
</tr>
<tr>
<td>10</td>
<td>0.244</td>
</tr>
<tr>
<td>25</td>
<td>0.612</td>
</tr>
<tr>
<td>50</td>
<td>1.303</td>
</tr>
<tr>
<td>100</td>
<td>2.773</td>
</tr>
</tbody>
</table>

**Limitations of the Procedure**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out according to the instructions of the package insert instructions and with adherence to good laboratory practice.

2. The results obtained from this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

3. Sample sera demonstrating gross lipemia, gross hemolysis, or turbidity should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

4. Inter-Assay Precision

- Between-run variability is shown below:
- Within-assay variability is shown below:

5. Specificity

The following hormones were tested for cross-reactivity and found with zero reaction:

<table>
<thead>
<tr>
<th>MATERIAL TESTED</th>
<th>TEST CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>20 g/L</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>8.0 g/L</td>
</tr>
<tr>
<td>Human IgG</td>
<td>30 g/L</td>
</tr>
</tbody>
</table>

**References**


