C-Reactive Protein (hs-CRP) Test System
Product Code: 3125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of C-Reactive protein (CRP) concentration in Human Serum, or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

C-Reactive Protein has traditionally been used to diagnose and monitor acute inflammation. It was named as such for its ability to bind and precipitate nonspecifically to a wide variety of diseases. These include infections by bacteria, acute phase of rheumatoid arthritis, abdominal abscesses and inflammation of the bile duct. High levels of CRP may also be found in patients with some viral infections, tuberculosis, acute infectious hepatitis, many other inflammatory disorders, burns and surgical trauma victims. Even though elevated levels of CRP are not indicative of any specific disease, the sudden rise of CRP does indicate an inflammatory process. CRP levels rise in circulation indicative of any particular disease, the sudden rise of CRP does

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place directly on the surface of the well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CRP antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme- labeled antibody and unconjugated antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{Ab}_\text{m}(\text{Biotin}) + \text{Ab}_\text{p} - \text{Ag} + \text{Anti-Ab}_\text{p} = \frac{\text{E}_{\text{imm}}}{\text{Ab}_\text{m}} \text{Ab}_\text{m}(\text{Biotin}) \text{Ab}_\text{p} \text{Ag} \text{Anti-Ab}_\text{p}
\]

where:

- \(\text{Ab}_\text{m}(\text{Biotin})\): Biotinylated monoclonal Antibody (Excess Quantity)
- \(\text{Ab}_\text{p}\): Native Antigen (Variable Quantity)
- \(\text{E}_{\text{imm}}\): Enzyme conjugated with a binding protein (Excess Quantity)
- \(\text{K}_\text{d}\): Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reactivity of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
\text{Ab}_\text{m}(\text{Biotin}) + \text{Ab}_\text{p} - \text{Ag} + \text{Streptavidin}_{\text{E}} = \text{Immolized complex}
\]

Streptavidin_{\text{E}}: Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by incubation with an excess of an antibody specific to CRP. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown specimen's activity can be correlated with CRP concentration.

4.0 REAGENTS

Materials Provided:
A. CRP Calibrators – 1ml/vial - Icons A-F
B. CRP Antigen at levels of 50(A), 0.5(B), 2.0(C), 5.0(D), 15(E) and 30(F) mg/l. Store at 2-8°C. A preservative has been added.
C. Serum Diluent
D. Enzyme Reagent  = Streptavidin immobilized on well. Mix and label accordingly.
E. Anti-CRP HRP in buffer, dye, and preservative. Store at 2-8°C.
F. Streptavidin Coated Plate – 96 wells - Icon

5.0 PRECAUTIONS

It is very important to dispense all reagents close to the bottom of the coated well. Before proceeding with the assay, bring all reagents, serum standards and patient samples to room temperature (20 - 27°C).

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimen shall be serum, plasma or urine. It is recommended in the collection of venipuncture samples should be collected in vacutainer tubes. For routine CRP determinations, soak the specimen in normal volumes, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to avoid interference. Avoid repetitive collection of the specimen.

Note 1: Do not use any specimen that has been refrigerated or frozen.

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in each test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends and detect deviation performance can indicate unnoticed change in experimental conditions or degradation of the reagents. Each laboratory should be used to determine the reason for the variations.

6.1 TEST PROCEDURE

Section), decant (tap and blot) or aspirate. If decanting, tap and blot the plate dry with absorbent paper. Avoid repetitive collection of the specimen.

8.0 REAGENT PREPARATION

1. Serum Diluent
   Dilute the serum diluent to 200µl in a suitable container with distilled or deionized water. Store at 2-8°C.

2. Wash Buffer
   Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2- 30°C, refrigerate if necessary and discard after 60 days. Store at 2-8°C.

3. Working Substrate Solution – Stable for one year.
   Note: For the preparation of the Working Substrate Solution, use the clear vial for easy identification. Mix and label accordingly. Store at 2- 8°C.

4. Patient Sample Dilution (1:200)
   Dilute 0.1ml (10µl) of each patient specimen into 2ml of serum diluent. Cover and vortex or mix thoroughly by inversion and discard after 60 days. Store at 2-8°C.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External use in Humans or Animals

6.0 SPECIMEN COLLECTION AND PREPARATION

As previously mentioned, a fasting morning serum sample should be obtained. Avoid repetitive collection of the specimen.

Note: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

Note: THE CALIBRATORS ARE READY TO USE.

7.0 QUALITY CONTROL

The specimens shall be serum, plasma or urine. It is recommended in the collection of venipuncture samples should be collected in vacutainer tubes. For routine CRP determinations, soak the specimen in normal volumes, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anticoagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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Note 1: Do not use any specimen that has been refrigerated or frozen.

It is very important to dispense all reagents close to the bottom of the coated well. Before proceeding with the assay, bring all reagents, serum standards and patient samples to room temperature (20 - 27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microtubes' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back in the container (avoiding air bubbles) to dispense the wash. Do not use reagents beyond the kit expiration date.

2. Pipette 0.025 ml (25µl) of the appropriate serum mixture, diluted control or specimen (see Sample Preparation Section) into the assay wells. Avoid air bubbles.

3. Add 0.100 ml (100µl) of the CRP Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well. Before proceeding with the assay, bring all reagents, serum standards and patient samples to room temperature (20 - 27°C).

Note: Use a multichannel pipet to quickly dispense the Enzyme Reagent. If the dilution is taking to more than a few minutes.

4. Swirl the microtubes gently for 20-30 seconds to mix and cover.

5. Incubate 15 minutes at 2-8°C.

6. Discard the contents of the microtube by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper. Avoid repetitive collection of the specimen.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Replace two (2) aliquots for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to aspirate the wash. Do not bring the wash bottle to the point of suction.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Do NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

9. Incubate at room temperature for fifteen (15) minutes.

10. Stop the reaction with 0.010ml (10µl) of 3N HCl. Store at 2-8°C.

11. Read the absorbance in each well at 450nm (using a reference blank) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Note: Always add reagents in the same order to minimize
1. Maximum Absorbance (Calibrator 'F') = >1.3

In order for the assay results to be considered valid the Q.C. PARAMETERS prepared with each assay.

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Calibrator 'F') = >1.3
2. Maximum Absorbance (Calibrator 'A') = > 0.1

3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to ensure reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay variability.
3. Highly hemolyzed, hemoglobinized or grossly contaminated specimen(s) should not be used.
4. If the value (µg/ml) is used, it is recommended to report the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, termination may be affected by the presence of other substances. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CRP concentrations above 30µg/ml may be further diluted (for example 1:50) with serum or diluent and re-assayed. The sample concentration is obtained by multiplying the result by the dilution factor (50).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device maintenance.
12. Risk Analysis - required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. The specificity of the method, the population tested and the per centile of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer. Only an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within-assay precision of the hsCRP AccuBind® ELISA Test System were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Within Assay Precision (Values in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Level 1</td>
<td>10</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The hsCRP Microplate Elisa Procedure has a sensitivity of 0.014 µg/ml. The sensitivity was ascertained by determining the variability of the 0 µg/ml calibrator and using 0 (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The hsCRP AccuBind® ELISA Test System was compared against a predicate automated hsCRP method. Biological samples (n=167) from population (symptomatic and asymptomatic) were used. The values ranged from 0 – 22 µg/ml. The correlation is presented in Table 4.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Mean</td>
</tr>
<tr>
<td>Correlation Analysis</td>
<td>Regression Coefficient</td>
</tr>
<tr>
<td>Monobind (x)</td>
<td>3.70</td>
</tr>
<tr>
<td>Reference (y)</td>
<td>3.94</td>
</tr>
</tbody>
</table>

14.4 Specificity

The cross-reactivity of the hsCRP AccuBind® ELISA Test System to selected substances was evaluated by adding the interfering substance to a pooled serum matrix at various concentrations, the cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of CRP needed to produce the same absorbance.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>ND</td>
</tr>
<tr>
<td>Lipids</td>
<td>ND</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>ND</td>
</tr>
<tr>
<td>Human IgG</td>
<td>ND</td>
</tr>
</tbody>
</table>

14.5 High Dose Hook Effect

The test will not be affected by CRP concentrations up to 5000 µg/ml in serum or plasma. However, samples expected to be over 30 µg/ml should be further diluted in working serum diluent.

15.0 REFERENCES

2. Centers for Disease Control/ NIH manual, Biosafety in Microbiological and Biomedical Laboratories (1994).

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<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Well</th>
<th>Abs</th>
<th>Mean Abs (B)</th>
<th>Conc (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td></td>
<td>0.045</td>
<td>0.045</td>
<td>0</td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td>0.048</td>
<td>0.048</td>
<td>0</td>
</tr>
<tr>
<td>C1</td>
<td></td>
<td>0.029</td>
<td>0.029</td>
<td>0</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td>0.122</td>
<td>0.122</td>
<td>0.5</td>
</tr>
<tr>
<td>E1</td>
<td></td>
<td>0.359</td>
<td>0.366</td>
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<td>F1</td>
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<td>0.687</td>
<td>0.683</td>
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<td>0.863</td>
<td>5.0</td>
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<td>H1</td>
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<td>0.864</td>
<td>0.864</td>
<td>5.5</td>
</tr>
<tr>
<td>I1</td>
<td></td>
<td>1.000</td>
<td>1.000</td>
<td>15.0</td>
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<tr>
<td>J1</td>
<td></td>
<td>1.112</td>
<td>1.112</td>
<td>15.0</td>
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<tr>
<td>K1</td>
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<td>2.611</td>
<td>2.564</td>
<td>30.0</td>
</tr>
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<td>L1</td>
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<td>2.517</td>
<td>2.517</td>
<td>30.0</td>
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<tr>
<td>M1</td>
<td></td>
<td>0.952</td>
<td>0.952</td>
<td>5.6</td>
</tr>
<tr>
<td>N1</td>
<td></td>
<td>2.162</td>
<td>2.115</td>
<td>19.8</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>2.000</td>
<td>2.000</td>
<td>20.0</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>2.218</td>
<td>2.201</td>
<td>21.9</td>
</tr>
</tbody>
</table>

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>1.6</td>
<td>0.13</td>
<td>8.2%</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>6.4</td>
<td>0.73</td>
<td>11.6%</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>12.1</td>
<td>1.09</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.