Mouse c-reactive protein (CRP) ELISA

Enzyme Immunoassay for the quantitative determination of mouse c-reactive protein (CRP) in serum.

REF  LD51031

12 x 8

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

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Enzyme Immunoassay for the Quantitative Determination of Mouse C-Reactive Protein (CRP) in Serum

INTRODUCTION
CRP is an acute phase protein in mice that is elevated in serum as a result of injury, infection or disease. It is reported that mouse CRP titer increases ~20 fold in mouse serum as a result of exposure to endotoxin. Studies at indicate that normal serum levels of CRP are approximately 500 ng/ml and that levels can exceed 5000 ng/ml following challenge with lipopolysaccharide. Measurement of CRP provides a convenient marker of inflammation and disease.

PRINCIPLE OF THE TEST
The mouse CRP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-mouse CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-mouse CRP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and 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-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of CRP is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- Anti-mouse CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (0.20 ml, lyophilized), containing mouse CRP (concentration and dilution instructions are listed on the vial label)
- Wash Buffer (20x stock, 50 ml)
- Diluent (30 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:
- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer.
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm

- A microtiter plate reader capable of measuring absorbance at 450 nm.
- Graph paper (PC graphing software is optional)

STORAGE OF Test Kit
The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase, provided that the components are stored as described above.

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum or plasma samples should be diluted ~500 fold with diluent in order to obtain values within the standard range.

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 mouse CRP standard is comprised of lyophilized mouse serum of known CRP concentration. The CRP content was determined by reference to purified mouse CRP.
1. Reconstitute the lyophilized mouse CRP reference standard by addition of 200 μl of de-ionized or distilled water. Mix gently several times over a period of 5-10 minutes. The concentration of CRP in the reconstituted stock is indicated on the vial label.
2. Label 5 polypropylene tubes as 125, 62.5, 31.25, 15.6, and 7.8 ng/ml
3. Into the tube labeled 125 ng/ml, pipette the volume of diluent detailed on the CRP reference standard vial label. Then add the indicated volume CRP standard (shown on the vial label) and mix gently. This provides the 125 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 62.5, 31.25, 15.6, and 7.8 ng/ml
5. Pipette 250 μl of the 125 ng/ml CRP standard into the tube labeled 62.5 ng/ml and mix. This provides the working 62.5 ng/ml CRP standard.
6. Prepare a 31.25 ng/ml standard by diluting and mixing 250 μl of the 62.5 ng/ml standard with 250 μl of diluent in the tube labeled 31.25 ng/ml. Similarly prepare the 15.6, and 7.8 ng/ml standards by serial dilution.

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

SAMPLE PREPARATION
General Note: We find that CRP is present in mouse serum at concentrations ranging from 500 to 5000 ng/ml or greater. In order to obtain values within the range of the standard curve we suggest that samples be diluted...
100 fold using the following procedure for each sample to be tested (please note that this is only a suggestion, you may have to determine the optimal dilution of your samples empirically):
1. Dispense 297 μl of diluent into separate tubes.
2. Pipette and mix 3 μl of the serum sample into the tube containing 297 μl of diluent. This provides a 100 fold diluted sample.
3. Repeat this procedure for each sample to be tested

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture by flicking the plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 6 times with 1x wash solution. This may performed using either a plate washer (400 μl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
7. Add 100 μl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual wash solution.
11. Dispense 100 μl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 μl of Stop Solution to each well.
14. Gently mix. It is important to make sure that all the blue color changes to yellow.
15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS
1. Calculate the average absorbance values (A₄₅₀) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of CRP in the serum sample.
5. If available, PC graphing software should be used for the above steps. We find that good a good fit of the data are obtained to a two site binding equation.
6. If the OD₄₅₀ values of samples fall outside the standard curve when tested at a dilution of 100, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE
A typical standard curve with optical density readings at 450nm on the Y axis against CRP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>CRP (ng/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>3.236</td>
</tr>
<tr>
<td>62.5</td>
<td>2.458</td>
</tr>
<tr>
<td>31.25</td>
<td>1.427</td>
</tr>
<tr>
<td>15.63</td>
<td>0.784</td>
</tr>
<tr>
<td>7.81</td>
<td>0.543</td>
</tr>
</tbody>
</table>

REFERENCES
2. Patterson, LT and Higginbotham, ED. Mouse C-Reactive Protein and Endotoxin-Induced Resistance. J Bacteriology. 90: 1520-1524 (1965)
LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.