RABBIT C-REACTIVE PROTEIN (CRP) ELISA
Life Diagnostics, Inc., Catalog Number: CRP-10

Rabbit C-Reactive Protein (CRP) ELISA

INTRODUCTION

CRP is an acute phase protein in rabbits that is elevated in serum as a result of injury, infection or disease. It is reported that CRP levels can increase several hundred fold in rabbit serum during the acute phase response.[1-4] Measurement of CRP therefore provides a convenient marker of inflammation and disease.

PRINCIPLE OF THE TEST

The rabbit CRP test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-rabbit CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rabbit 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-rabbit CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (200 µl, lyophilized), containing rabbit CRP (concentration and dilution instructions are listed on the vial label), **Store at -20°C**.
- 10x Diluent, 25 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- 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

1. **The lyophilized reference standard should be stored at minus 20°C for optimum stability** (it can be safely shipped at 2-8°C).
2. The remainder of the 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.
3. 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 samples should be diluted ~1000 fold with 1x diluent in order to obtain values within the standard range.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

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 rabbit CRP standard is comprised of lyophilized rabbit serum of known CRP concentration. The CRP content was determined by reference to purified rabbit CRP prepared at Life Diagnostics, Inc.

1. Reconstitute the lyophilized rabbit CRP reference standard by addition of 200 µl of deionized 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 8 polypropylene tubes as 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 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 1x diluent into the tubes labeled 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 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. Similarly prepare the 31.25, 15.63, 7.81, 3.91 and 1.95ng/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 of reconstitution) if future use is intended.

SAMPLE PREPARATION

CRP is present in rabbit serum at concentrations ranging from less than 100 ng/ml to several hundred µg/ml. In order to identify the optimum dilution for a particular sample set, we suggest that a limited number of samples be tested as singlets at a dilution of 1000 fold side by side with the 250 and 0 ng/ml standards. Based

on these results, an appropriate dilution factor for the remaining samples might be estimated. A 1000 fold dilution of serum samples may be obtained as follows:

1. Using a precision micropipette, pipette and mix 1.0 µl of the serum sample into a tube containing 999 µl of 1x diluent. This provides a 1000 fold diluted sample.
2. Repeat this procedure for each sample to be tested.

Please dilute serum samples a minimum of 10 fold.

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 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 5 times with wash solution. This may be performed using either a plate washer or 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 water droplets.
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.

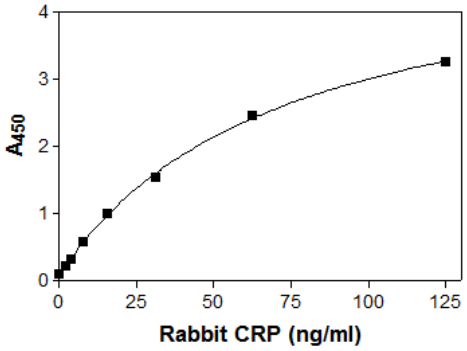
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) 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 concentration by the dilution factor to determine the actual concentration of CRP in the serum sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of diluted samples fall outside the standard curve when tested at a dilution of 1000, samples should be re-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.

CRP (ng/ml)	A_{450}
125	3.259
62.5	2.464
31.25	1.538
15.63	0.999
7.81	0.590
3.91	0.326
1.95	0.221
0	0.095



REFERENCES

1. Kushner I and Feldman G. Control of the acute phase response: Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. J. Exp. Med. 148:466-477 (1978)
2. Qian Z et. al. Targeting vascular injury using hantavirus-pseudotyped lentiviral vectors. Molecular Therapy 13:694-704 (2006)
3. Cenesiz S et. al. Effects of fluoride on C-reactive protein, adenosine deaminase, and ceruloplasmin in rabbit sera. Research report Flouride 41(1)52-56 (2008)
4. Ferrian S. et. al. Comparison of immune response to lipopolysaccharide of rabbit… Res Vet Sci. 94(3):518-25 (2013)

Rev 041015NC

For technical assistance please email us at
techsupport@lifediagnostics.com