HIGH SENSITIVITY C-REACTIVE PROTEIN ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00016

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

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

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PRINCIPLE OF THE ASSAY

The hsCRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay.[24] The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the 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 solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A 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 1N HCl changing the color to yellow. The concentration of CRP is directly proportional the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-CRP.
2. *Reference Standard Set (1.0 ml/vial)*
   Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/l CRP in phosphate buffer-BSA solution with preservatives; liquid, ready to use.
3. *hsCRP Sample Diluent (50 ml/vial)*
   Contains phosphate buffer-BSA solution with preservatives.
4. *CRP Enzyme Conjugate Reagent (12 ml/vial)*
   Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.
5. *TMB Reagent (11 ml/bottle)*
   Contains one-step TMB solution.
6. *Stop Solution (1 bottle, 11 ml/bottle)*
   Contains diluted hydrochloric acid (1N HCl).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 100 µl and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper

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 (18-25°C) before use.
2. ***Sample 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 µl of serum with 495 µl (0.495 ml) Sample Diluent. DO NOT DILUTE THE STANDARDS.***
3. Samples with expected CRP concentrations over 10 mg/l may be quantitated by further dilution (10 fold) of the 100-fold diluted solution with sample diluent (i.e., 10 µl of the 100-fold diluted sample to 90 µl sample diluent).

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

ASSAY PROCEDURE

1. ***Sample serum and control serum should be diluted 100 fold prior to use. See Reagent Preparation. PLEASE DO NOT DILUTE THE STANDARDS.***
2. Secure the desired number of coated wells in the holder.
3. Dispense 10 µl of UNDILUTED CRP standards, DILUTED specimens, and DILUTED controls into appropriate wells.
4. Dispense 100 µl of CRP Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix them completely.
6. Incubate at room temperature (18-25 °C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. DO NOT USE TAP WATER.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 100 µl TMB solution into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read absorbance at 450 nm with a microtiter well reader ***within 15 minutes.***

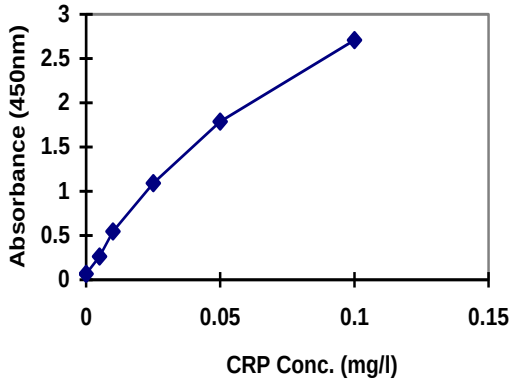
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) 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 (mg/l) 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 samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
5. Samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/l.
6. NOTE: Samples with CRP concentrations less than 0.1 mg/l should reported as "<0.1 mg/l CRP".

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against CRP concentrations shown on the X axis. **NOTE:** This standard 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.

CRP (mg/l)	Absorbance (450 nm)
0	0.066
0.005	0.264
0.010	0.457
0.025	1.092
0.050	1.788
0.100	2.710



TECHNICAL CONSULTATION

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