



EAGLE
BIOSCIENCES

C-Reactive Protein HS ELISA

Catalog Number: CRP31-K01

For Research Use Only. Not for use in diagnostic procedures.

v. 2.1 (18 OCT 24)

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INTENDED USE

The C-Reactive Protein High Sensitivity ELISA is intended for the quantitative determination of C-reactive protein (CRP) concentration in serum .

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY BACKGROUND

The C-Reactive Protein HS 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 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 1 N 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.

MATERIALS PROVIDED

- **Antibody-coated wells** (1 plate, 96 wells)
Microtiter wells coated with mouse monoclonal anti-CRP
- **Reference Standard Set** (1.0 mL/vial)
Contains 0 – 0.005 – 0.010 – 0.025 – 0.050 – and 0.100 mg/L CRP in serum based buffer with preservatives
- **hsCRP Sample Diluent** (50 mL/vial)
Contains phosphate buffer-BSA solution with preservatives.
- **CRP Enzyme Conjugate Reagent** (12 mL/vial)
Contains goat anti-CRP conjugated t horseradish peroxidase with preservatives
- **TMB Reagent** (11 mL/Bottle)
Contains one-step TMB solution
- **Stop Solution** (11mL/bottle)
Contains diluted hydrochloric acid (1N HCl)

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes 5 μ L, 10 μ L, 50 μ L, 100 μ L and 1.0 mL
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450nm
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper



WARNINGS AND CAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HGV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Avoid contact with 1 N HCl. 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 lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use.

STORAGE CONDITIONS

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

ASSAY PREPARATIONS

- All reagents should be allowed to reach room temperature (18°C – 25°C)before use.
- Serum should be diluted 100-fold prior to use. Prepare a series of small tubes (i.e 1.5mL microcentrifuge tubes) and mix 5 μ L of serum with 495 μ L (0.495mL) Sample Diluent.

DO NOT DILUTE THE STANDARDS

- Samples with expected CRP concentration over 10mg/L may be quantitated by further dilution (10 fold) of the 100-fold diluted solution with sample diluent.
(i.e., 10 μ L of the 100-fold diluted sample to 90 μ L sample diluent)

INSTRUMENTATION

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

ASSAY PROCEDURE

1. 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 μ L** of CRP Standards, **DILUTED** Specimens, and **DILUTED** controls into appropriate wells.
4. Dispense **100 μ L** of CRP Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18°C-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 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 μ L** 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 μ L** 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 450nm with microtiter well reader **within 15 minutes.**

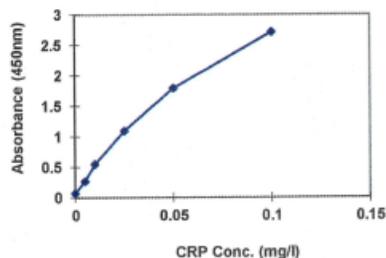
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD₄₅₀) 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 the 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 concentration greater than 10 mg/L should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/L.
6. NOTE: Samples with CRP concentration less than 0.1 mg/L should be reported as "<0.1mg/L CRP".

EXAMPLE STANDARD CURVE

Results of a typical standard run with absorbance 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





LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to a good laboratory practice.
2. For professional use only
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Samples may contain human anti-mouse antibodies (HAMA) that are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens. Nevertheless, complete elimination of this interference from all specimens cannot be guaranteed.
6. If the device fails to perform, use alternative procedure or consult manufacturer.\

ASSAY CHARACTERISTICS

Detection Limit

The minimum detectable concentration of the CRP ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.1 mg/L

Lower limit of C-Reactive Protein HS ELISA \cong 0.1 mg/L CRP; upper limit = 10 mg/L CRP

Specificity

The following analytes were tested for cross-reactivity:

Material Tested	Test Concentration
Bilirubin	50 mg/L
	100 mg/L
	230 mg/L
Hemoglobin	12 g/L
	25 g/L
	36 g/L
Triglyceride	2.5 g/L
	5.0 g/L
	7.5 g/L
Human IgG	5 g/L
	10 g/L
	25 g/L

No cross-reactivity was observed at all testing concentrations



WARRANTY INFORMATION

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.