EAGLE BIOSCIENCES

High Sensitivity C-Reactive Protein (hs-CRP) ELISA Assay Kit

Catalog Number:
HCR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.0 (29 NOV 23)

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INTENDED USE

The Eagle Biosciences High Sensitivity C-Reactive Protein (hs-CRP) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of C-Reactive Protein in human serum by an enzyme immunoassay. The Eagle Biosciences High Sensitivity C-Reactive Protein (hs-CRP) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

C-reactive protein (CRP) is a pentameric acute phase reactant that is synthesized by the liver. It's production is controlled primarily by interleukin-6. The serum CRP concentration may increase by up to 1000-fold with infection, trauma, surgery, and other acute inflammatory events. Chronic inflammatory disorders such as auto-immune diseases and malignancy can produce persistent high levels of serum CRP.
Traditionally, CRP has been used clinically for the diagnosis and monitoring of auto-immune and infectious disorders. Recent studies have shown that chronic inflammation is an important component in the development and progression of atherosclerosis. As a result, increased serum CRP concentration are positively associated with the risk of future coronary events.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microplate and another monoclonal antibody specific for a different region of CRP is conjugated to horse radish peroxidase (HRP). CRP from the sample and standards are allowed to bind to the plate, washed and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of CRP in the sample.
A set of standards is used to plot a standard curve from which the amount of CRP in samples and controls can be directly treated.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for research use only.
2. Practice good laboratory practices when handling kit reagents.
   This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
   - Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this it. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A calibrator curve must be established for every run.

6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) should be included in every run and fall within the acceptable ranges, as stated in the quality control certificate.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
15. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of CRP in human serum. The kit is not calibrated for the determination of CRP in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibratory A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as a sole basis for clinical diagnosis, and is strictly for research use only.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.
All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

SPECIMEN PRETREATMENT
Dilute serum samples 1:20 with calibrator A before use. Example: to 190 µL of calibrator A add 10 µL of serum sample (1:20)
*Do not dilute the standards and controls, they are ready for use.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipettes to dispense 20, 50, 100, 150 and 350 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)
6. Polypropylene/HDPE or disposable glass tubes for sample pre-treatment
7. Vortex Mixer

REAGENTS PROVIDED

1. Mouse Anti-CRP Antibody-Coated Break-Apart Well Microplate — Ready To Use

Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

2. Mouse Anti-CRP Antibody-Horseradish Peroxidase (HRP) Conjugate — Requires Preperation (x81)

Contents: Mouse Anti-CRP Antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 0.3 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:81 in assay buffer before use (eg. 25 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 150 µL of HRP in 12 mL of assay buffer. Discard any that is left over

3. CRP Calibrators — Ready To Use

Contents: Six vials containing CRP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of CRP.

Calibrator concentrations*: 0, 100, 400, 1000, 4000 and 10,000 ng/mL
* Approximate value – please refer to vial labels for exact concentrations.

Volume: Calibrators A-16 mL/vial:, B-F: 0.5 mL/vial
Storage: Refrigerate at 2–8°C.
Stability: 12 months in unopened vials or as indicated on label.

4. Controls — Ready to Use

Contents: Two vials containing CRP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of CRP. Refer to vial labels for the acceptable range.
Volume: 0.5 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label.

5. Wash Buffer Concentrate — Requires Preparation (x10)

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer — Ready To Use

Contents: One bottle containing protein-based buffer with a non-mercury preservative.
Volume: 40 mL/kit
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

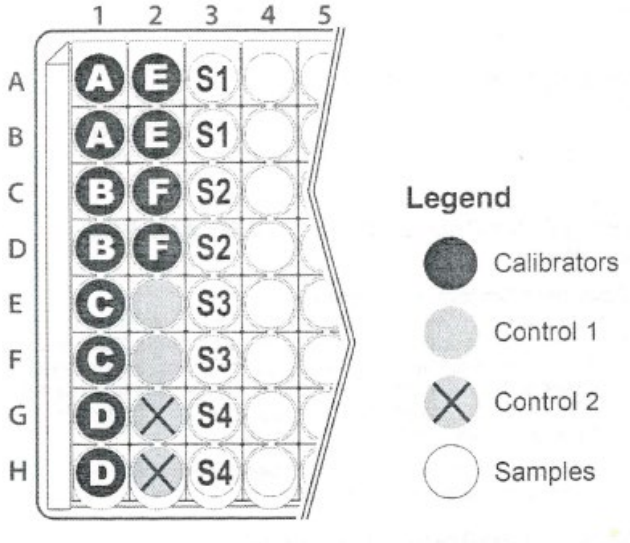
7. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DSMO containing buffer
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

8. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

Specimen Pretreatment: Dilute 1:20 with calibrator A before use

1. Prepare working solutions of the anti-CRP-HRP conjugate and wash buffer
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control and pre-treated specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 200 µL of the assay buffer into each well.
5. Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 30 minutes at room temperature.
6. Wash the wells 3 times each time with 300 µL/well of working wash buffer solution. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
7. Pipette 100 µL of the conjugate working solution into each well.
8. Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 15 minutes at room temperature.
9. Wash wells again in the same manner as step 6
10. Pipette 100 µL of TMB Substrate into each well at timed intervals.
11. Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 15 minutes at room temperature.
12. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10. Mix thoroughly by gently tapping the plate.
13. Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls and samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a serum reads greater than 10,000 ng/mL, then dilute it with the calibrator A at a dilution of no more than 1:10 from the original 1:20 dilution serum (or 1:200 from neat serum). The result obtained must be multiplied by the dilution factor.

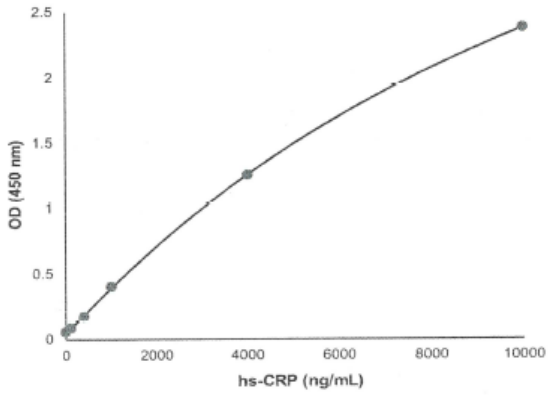
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	0.054	2	0
B	0.085	4	100
C	0.174	7	400
D	0.406	17	1000
E	1.254	53	4000
F	2.376	100	10000
Unknown	1.021	-	3072

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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