Product Manual

Human C-Reactive Protein (CRP) ELISA Kit

Catalog Number

STA-392 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

C-Reactive Protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. CRP belongs to the pentraxin family of calcium dependent ligand-binding plasma proteins. Human CRP binds with highest affinity to phosphocholine residues, but it also binds to a variety of other autologous and extrinsic ligands, and it aggregates or precipitates the cellular, particulate, or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles, and apoptotic cells. Extrinsic ligands include many glycan, phospholipid, and other constituents of microorganisms, such as capsular and somatic components of bacteria, fungi, and parasites, as well as plant products. CRP has been associated with cardiovascular disease, atherosclerosis and inflammation, and other human diseases.

Cell Biolabs' Human CRP ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human CRP in plasma, serum or other biological fluid samples. The kit has detection sensitivity limit of 1 ng/mL human CRP. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-362: Human ApoAI ELISA Kit
2. STA-363: Human ApoAII ELISA Kit
3. STA-364: Human ApoCI ELISA Kit
4. STA-365: Human ApoCII ELISA Kit
5. STA-366: Human ApoCIII ELISA Kit
6. STA-367: Human ApoE ELISA Kit
7. STA-368: Human ApoB-100 ELISA Kit
8. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
9. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
10. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human CRP Antibody Coated Plate (Part No. 239201): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human CRP Antibody (1000X) (Part No. 239202): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.

6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human CRP Standard (Part No. 239203): One 100 µL vial of 10 µg/mL Human CRP in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human CRP Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human CRP Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human CRP antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of human CRP standards in the concentration range of 0 to 80 ng/mL in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human CRP Standard (µL)	Assay Diluent (µL)	Human CRP (ng/mL)
1	8	992	80
2	500 of Tube #1	500	40
3	500 of Tube #2	500	20
4	500 of Tube #3	500	10
5	500 of Tube #4	500	5
6	500 of Tube #5	500	2.5
7	500 of Tube #6	500	1.25
8	0	500	0

Table 1. Preparation of Human CRP Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Note: Normal baseline levels of circulating CRP are low (<10 µg/mL*), but may increase 10,000-fold within hours of inflammation induced by infection or injury.*

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC. Dilute samples in PBS containing 0.1% BSA as needed.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC. Dilute samples in PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months.

Assay Protocol

1. Prepare dilutions of plasma, serum or other biological fluid samples in PBS containing 0.1% BSA.
2. Add 100 µL of human CRP unknown sample or standard to the Anti-Human CRP Antibody Coated Plate. Each CRP unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-Human CRP antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human CRP ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

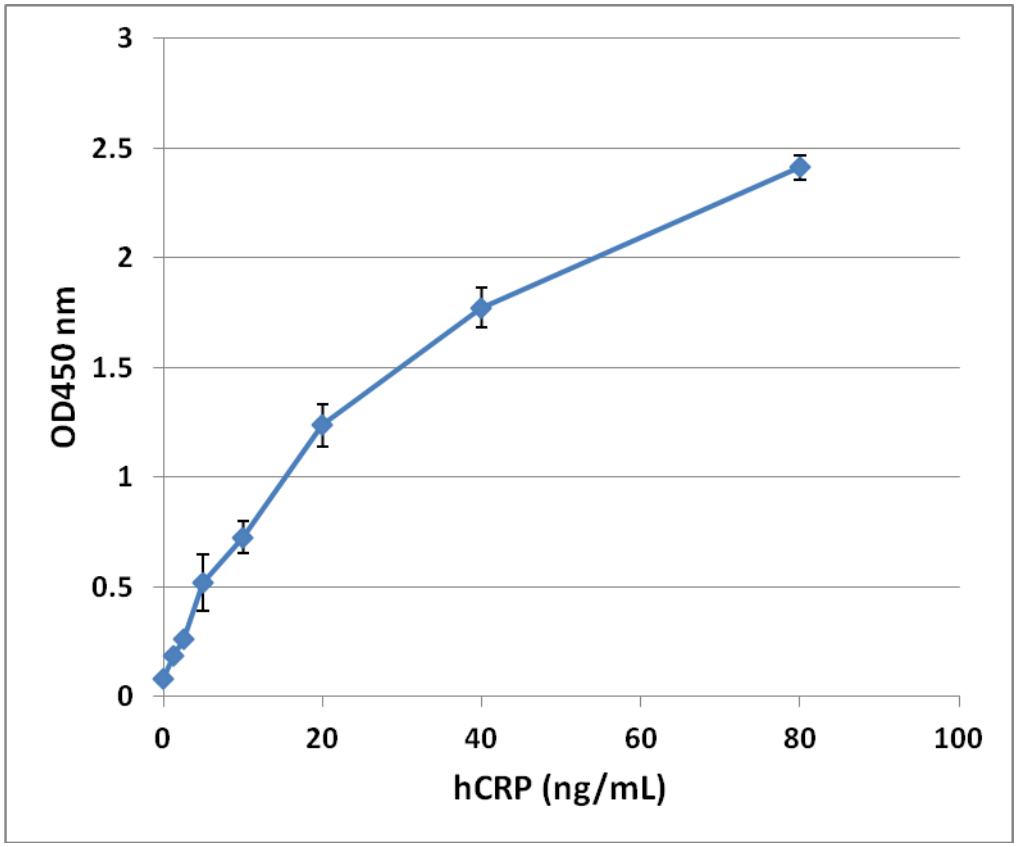

Figure 1: Human CRP ELISA Standard Curve.

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Recent Product Citations

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