Human hsCRP ELISA Kit

Enzyme Immunoassay for the quantitative high sensitive determination of human C-Reactive Protein in serum and plasma

Catalog number: ARG80876

distributed in the US/Canada by:
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INTRODUCTION

C-Reactive Protein (CRP) is an acute-phase protein, produced exclusively in the liver. Interleukin- 6 is the mediator for the synthesis by the hepatocytes of CRP, a pentamer of approximately 120.000 Daltons. CRP is present in the serum of normal persons at concentrations ranging up to 5mg/l. A series of prospective studies provide consistent data documenting that mild elevation of baseline levels of CRP among apparently healthy individuals is associated with higher long term risk for future cardiovascular events. This predictive capacity of CRP is independent of traditional cardiovascular risk factors and offers a prognostic advantage over measurement of lipid alone. Inflammatory markers specifically CRP may help to identify those who would benefit most from these pharmacological intervention. CRP is the novel and evolving biomarker which provides a most useful predictive indicator for subsequent cardiovascular events. This test should not be used for assessment of acute inflammation but should be ordered to evaluate CVD (Cardiovascular Disease) risk in apparently healthy individuals who have not had recent infection or other serious illness.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CRP has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CRP present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-CRP antibody conjugate is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is

added to the wells and color develops in proportion to the amount of CRP bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of CRP in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	12 ml (ready to use)	4°C
Standards 0-4 (0, 0.4, 1, 5, 10 µg/ml)	0.2 ml	4°C
5X Dilution buffer	40 ml	4°C
20X Wash buffer	50 ml	4°C
Substrate (TMB)	15 ml	4°C
Stop solution	12 ml	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially

while handling blood or body fluid samples.

- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer or 5X Dilution buffer, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Lipemic and/or haemolysed samples can cause false results so Lipemic and/or haemolysed samples should be avoided.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X wash buffer with distilled water to yield 1X wash buffer.

- 1X Dilution Buffer: Dilute 5X Dilution buffer with distilled water to yield 1X Dilution buffer.
- Standards: Dilute 1:100 with 1X Dilution buffer. (10µl of each standards + 990µl 1X Dilution Buffer, mix thoroughly.)
- Sample: The patient samples are diluted 1:1000 with 1X Dilution buffer in two consecutive steps.
    1. 1:100 diluted: 10µl of each samples + 990µl 1X Dilution Buffer, mix thoroughly.
    2. 1:1000 diluted: 50µl of 1:100 diluted sample from step 1 + 450µl 1X Dilution Buffer, mix thoroughly.

    Note: do not store the diluted samples for more than 8 hours.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of diluted standards and samples into each well.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the well for 1-2 min. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl HRP-antibody conjugate into each well. Incubate for 30

minutes at RT.

6. Aspirate and wash wells as step 4.
7. Add 100 µl of TMB Substrate to each well. Incubate for 10 minutes at room temperature in dark.
8. Add 50 µl Stop solution into each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

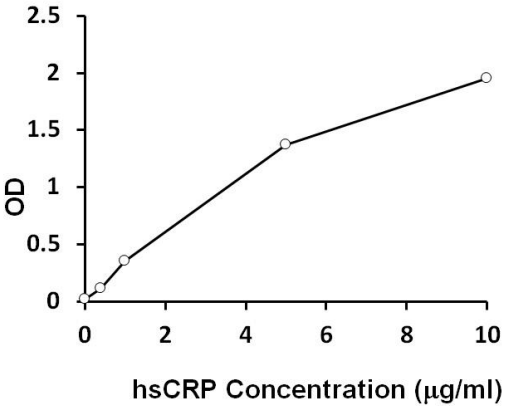
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimal detectable concentration is approximately 0.02 µg/ml.

Specificity

The human hsCRP ELISA kit recognizes natural and recombinant human CRP. No cross-reactivity was observed with following factors, prepared at 1 µg/ml in sample diluent: human pentraxin 2, pentraxin 3; monomeric CRP; rat CRP.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.1% and the CV value of inter-assay precision was 6.13%.

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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.

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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.