ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

High-sensitive Human C-Reactive Protein (CRP) ELISA Kit

(Catalog Number: 31220)

For the quantitative determination of human C-reactive protein concentrations in serum or plasma

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INTRODUCTION

C-reactive protein (CRP) is a circulating protein mainly secreted from the liver. This acute phase protein consists of five identical non-glycosylated subunits of 23 kDa, that give rise to a symmetrically arranged globular protein with molecular weight of approximately 120 kDa[1]. It has long been recognized that CRP is closely related to immunology, inflammation and host defense. As a result, it has been used as an inflammatory marker. However, the development of high-sensitivity CRP (hsCRP) ELISA had addressed its role in other clinical issues. There is accumulating evidence suggesting the important role that CRP plays in mediating cardiovascular diseases (CVD) and type 2 diabetes[2-4]. Normally CRP is present only in a trace amount in circulation (<1 μg/mL)[5,6] but can increase over 1,000-fold under acute inflammatory state. Individual with blood CRP levels <1 μg/mL, 1-3 μg/mL and >3 μg/mL is considered to have low, moderate and high risk, respectively, of CVD and myocardial infraction[7]. Therefore, blood CRP level has become a promising measure of CVD risk[8,9].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against human CRP. The microtiter plate is pre-coated with a monoclonal antibody specific for human CRP. Standards and samples are pipetted into the wells and any human CRP present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked monoclonal antibody specific for human CRP is added to the wells. After wash step to remove any unbound reagents, an HRP substrate solution is added and color develops in proportion to the amount of human CRP bound initially. The assay is stopped, and the optical density of the wells is determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured human CRP, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human CRP ELISA kit is designed for quantification of human CRP in serum and plasma.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter strips (96 wells), coated with a mouse monoclonal antibody against human CRP, sealed
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, a mouse monoclonal antibody against human CRP conjugated with horseradish peroxidase, 0.12 mL
5. Human CRP standard solution, 100 ng/mL of native human CRP in a buffered protein base solution, 0.3 mL
6. Substrate solution, 12 mL, ready for use

7. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

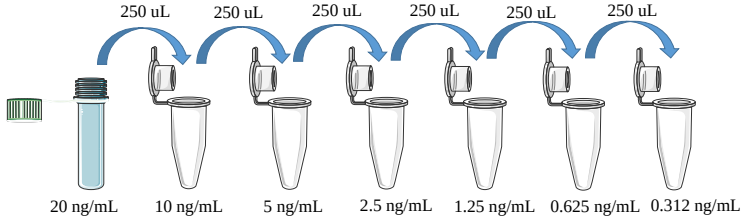
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1× Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human CRP Standards: Initially, prepare 20 ng/mL standard by diluting 100 µL of 100 ng/mL standard stock in 400 µL of 1X assay buffer**.** Prepare serially diluted standards using 1×Assay buffer as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL).

Sample Preparation:

Serum or plasma sample generally requires a **200-fold** dilution in this assay. A suggested dilution step is to add 5 µL of sample to 995 µL of 1×Assay buffer. If a sample has a CRP level greater than the highest standard, the sample should be diluted further, and the assay should be repeated. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standards and samples to each well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µL of Stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

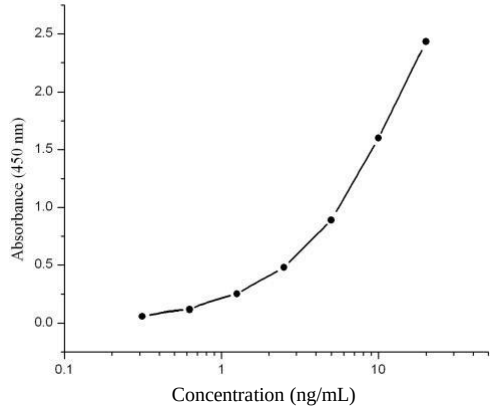
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human CRP concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human CRP concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

CRP (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.066	0
0.312	0.125	0.059
0.625	0.183	0.117
1.25	0.317	0.251
2.5	0.545	0.479
5	0.955	0.889
10	1.665	1.599
20	2.498	2.432

Human CRP standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of CRP that can be detected by this assay is 0.312 ng/mL.

B. Specificity

The antibody pair used in this assay is specific to human CRP and does not cross-react with mouse and rat CRP, and other cytokine or hormone molecules.

C. Precision

Intra-assay C.V.: 4.3%.
Inter-assay C.V.: 5.9%.

D. Recovery

The recovery of the assay was determined by adding various amounts of CRP to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 98%.

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SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation