



Antibody and Immunoassay Services

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Rapid high-sensitive human C-Reactive Protein (CRP) Immunoassay kit

Catalogue Number: 31222

For the quantitative determination of human C-Reactive Protein concentrations in serum and plasma samples.

This package insert must be read in its entirety before using this product.
Use only the current version of product data sheet enclosed with the kit.

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Version: 1.0

**FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES**

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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 defence, 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 presenting only in a trace amount in circulation ($<1 \mu\text{g/ml}$)⁵⁻⁶ but can increase over 1,000-fold under acute inflammatory state. Individual with blood CRP levels $<1 \mu\text{g/ml}$, $1-3 \mu\text{g/ml}$ and $>3 \mu\text{g/ml}$ is considered to have low, moderate and high risk, respectively, of CVD and myocardial infarction.⁷ Therefore, blood CRP level has become a promising measure of CVD risk.⁸⁻⁹

PRINCIPLE OF THE ASSAY

This assay is a sandwich ELISA designed for the quantitative detection of human CRP in samples in 1 hour. A mouse monoclonal antibody specific to human CRP has been pre-coated onto a micro-titre plate. Standards and samples are added into the wells and any human CRP present is sandwiched by the immobilised antibody and a second horseradish peroxidase (HRP)-linked monoclonal antibody specific to human CRP that is co-incubated with the samples. After wash step to remove any unbound substances, an HRP substrate solution is added and colour develops in proportion to the amount of human CRP bound initially. The assay is stopped and the optical density of the wells 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 Rapid Human CRP ELISA kit is designed for quantification of human CRP in serum and plasma samples.

MATERIALS PROVIDED

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

1. Micro-titre strips (96 wells)-Coated with a mouse monoclonal antibody against human CRP, sealed.
2. 10×Wash buffer-20 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.
8. Plate cover- 1.

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 deionised water.
7. Horizontal micro-plate shaker capable of 600 rpm.

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 microplate, 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 (20 ml) with 180 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, 200 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:

Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
100 ng/ml stock	-	100 ng/ml
100 µl of 100 ng/ml	100 µl	50 ng/ml
100 µl of 50 ng/ml	100 µl	25 ng/ml
100 µl of 25 ng/ml	100 µl	12.5 ng/ml
100 µl of 12.5 ng/ml	100 µl	6.25 ng/ml
100 µl of 6.25 ng/ml	100 µl	3.12 ng/ml
100 µl of 3.12 ng/ml	100 µl	1.56 ng/ml

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

Sample preparation

Serum or plasma sample is generally required a 100-fold dilution in this assay.

A suggested dilution is to add 10 µl of sample to 990 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.

ASSAY PROCEDURE

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

1. Add 10 μl of standard or sample per well.
2. Add 100 μl of the 1 \times Detection antibody solution to each well, seal the plate with a plate cover. Incubate at room temperature for 30 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
3. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 μl of 1 \times Wash buffer to each well and incubate for 1 minute. Discard the 1 \times 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.
4. Add 100 μl of Substrate solution to each well. Incubate at room temperature for 15 minutes. **Protect from light.**
5. Add 100 μl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
6. Measure absorbance of each well at 450 nm immediately.

CALCULATION

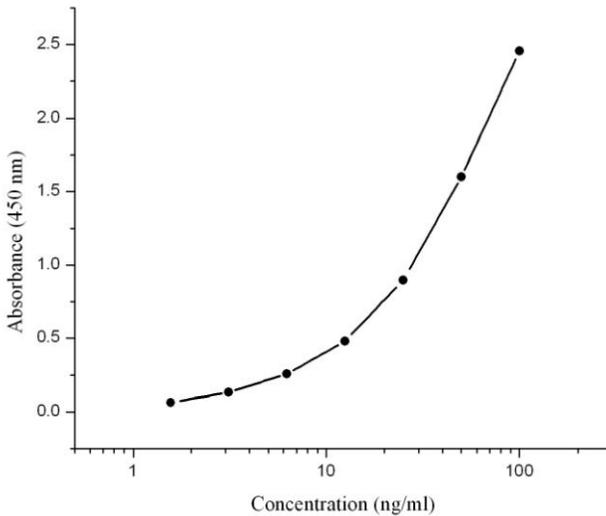
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human CRP concentrations (x-axis). The best fitting line can be generated with any curve-fitting software, any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human CRP concentration of samples from the 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	0.055	0
1.56	0.119	0.064
3.12	0.188	0.133
6.25	0.312	0.257
12.5	0.535	0.480
25	0.952	0.897
50	1.656	1.601
100	2.510	2.455

Human CRP standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity:

The lowest level of human CRP that can be detected by this assay is 1.56 ng/ml.

B. Specificity:

The antibody pair used in this assay is specific to human CRP and does not cross-react with mouse or rat CRP, or other cytokine and hormone molecules including human resistin, TNF α , ANGPTL4, insulin, leptin and IL6.

C. Precision

Intra-assay CV: 4.7%.

Inter-assay CV: 6.3%.

D. Recovery

The recovery of the assay was determined by adding various amounts CRP to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 95%.

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SUMMARY OF ASSAY PROCEDURE

Add 10 μ l of Standard or sample per well.
↓
Add 100 μ l of 1 \times Detection antibody solution to each well.
↓
Incubate at room temperature for 30 minutes (600 rpm).
↓
Aspirate and wash each well 3 times.
↓
Add 100 μ l of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 μ l of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation