Human C-Reactive Protein (CRP)

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55114-96

www.zellx.de

Sample Types Validated for:

Serum and Plasma

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Introduction

Background

C-reactive protein (CRP) belongs to the pentraxin family of proteins. It is synthesized by the liver and its level increases in response to inflammation. IL-6 primarily induces the transcription of CRP during the acute phase of an inflammatory/infectious disease. CRP is an acute-phase reactant protein and is widely used as a biomarker for systemic inflammation and tissue injury as its level rises and falls rapidly with the onset and eradication of the inflammatory stimulus, respectively.

CRP specifically binds to phosphocholine (PCh) residues of polysaccharides on many microbial pathogens and on apoptotic/necrotic cell membranes. Being recognized by the C1q complex of the complement system, the PCh-bound CRPs efficiently initiate the activation of the system, leading to the elimination of foreign pathogens. The binding of CRP to PCh on damaged cells facilitates the clearance of apoptotic/necrotic host cells, and contributes to the restoration of normal structure and function of injured tissue. Despite its anti-inflammatory properties, CRP can also exert pro-inflammatory properties when it is activated by autoantibodies, displaying the phosphocholine arm in auto-immune diseases.

The binding of CRP to Fc receptors FcγRI and FcγRIIa mediates the interaction of damaged cells or particles with phagocytic cells leading to phagocytosis of the cells or particles. The function of CRP in eliminating foreign pathogens and damaged cells through recruitment of the complement system and phagocytic cells makes CRP a critical molecule in the frontline of innate host defense. In response to infection, cell damage or tissue injury, the serum CRP concentration may increase by up to 1000 fold. Elevated CRP levels have been reported in patients with infection, chronic inflammatory disorders, myocardial infarction, ischemia/reperfusion injury, atherosclerosis, cancer, pulmonary disorders, metabolic syndrome and depression.

Assay principle

The ZellX® CRP Immunoassay kit is a competitive ELISA assay designed to quantitatively measure the amount of CRP present in serum and plasma**.** A CRP stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

The kit includes a 96-well plate that has been pre-coated with a mouse anti-CRP antibody. A peroxidase-conjugated CRP monoclonal antibody is pipetted into a clear microtiter plate coated with a monoclonal antibody to capture CRP present in the sample. After adding the samples and standards and subsequently 2 hours of incubation, the substrate is added to react with the bounded CRP-conjugated antibody. After stopping the reaction, the intensity of the generated color can be measured at 450 nm.

This kit uses the CRP Standard solutions calibrated to the 1st WHO International Standard National Institute for Biological Standards and Control (NIBSC).

General information

Materials supplied in the Kit

Component	*Quantity*
CRP Standard (400 ng/mL)	40 µL
CRP Conjugate	5 mL
Assay Buffer Concentrate (5x)	16 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

The CRP Standard is purified from a human source and, as such, should be treated as potentially hazardous material. Proper safety procedures must be followed.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.

- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The color of silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers which contain sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Wash Buffer Conc. can be stored at room temperature for up to 3 months.

Sample preparation

The assay has been validated for the measurement of free CRPs in both human plasma and serum samples. This assay has low or no reactivity to rodent CRP. Samples containing visible particulate should be centrifuged prior to conducting the assay.

Serum and plasma samples must be diluted ≥ 1:10 with diluted Assay Buffer prior to performing the assay. Typical CRP concentrations in human serum and plasma can be as high as mg/mL levels depending on disease state. We recommend diluting all samples 1:500 or 1:1000 fold in diluted Assay Buffer.

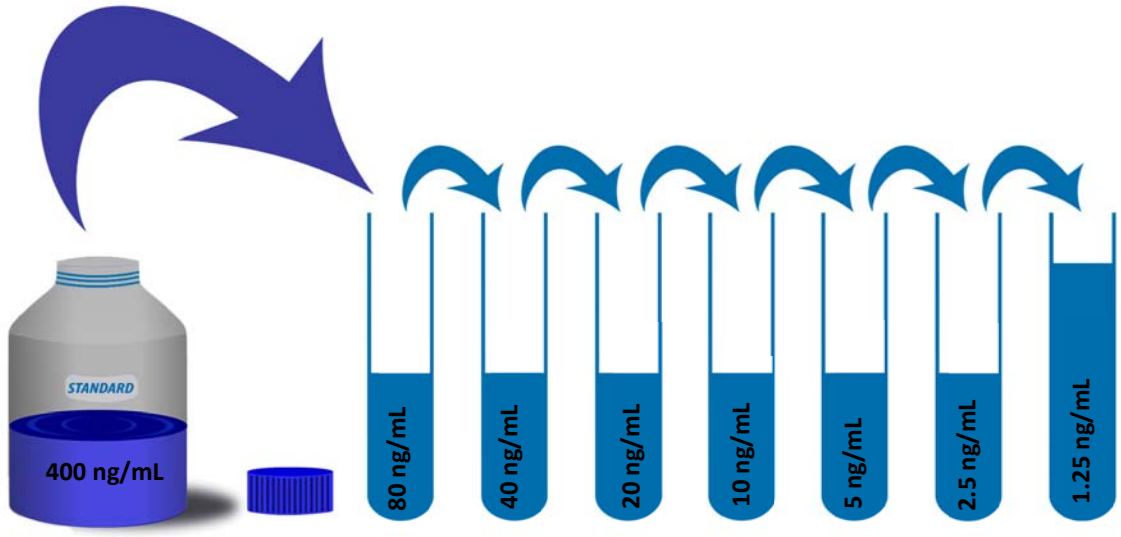
Any samples with concentrations outside the standard curve range should be diluted further with Assay Buffer, as appropriate, to obtain readings within the standard curve range.

All samples and standards must be used within 2 hours of preparation.

Standard preparation

- Prepare a 1:5 dilution of CRP Standard with Assay Buffer (mix 20 µL of standard with 80 µL of Assay Buffer), and label as the Standard No.7 (80 ng/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 ng/mL standard.

No.	*Concentration*	*Material needed*
Standard No.7	80 ng/mL	20 µL CRP Standard + 80 µL Assay Buffer
Standard No.6	40 ng/mL	50 µL Standard No.7 + 50 µL Assay Buffer
Standard No.5	20 ng/mL	50 µL Standard No.6 + 50 µL Assay Buffer
Standard No.4	10 ng/mL	50 µL Standard No.5 + 50 µL Assay Buffer
Standard No.3	5 ng/mL	50 µL Standard No.4 + 50 µL Assay Buffer
Standard No.2	2.5 ng/mL	50 µL Standard No.3 + 50 µL Assay Buffer
Standard No.1	1.25 ng/mL	50 µL Standard No.2 + 50 µL Assay Buffer
Standard No.0	0 ng/mL	50 µL Assay Buffer



All standards must be used within 2 hours of preparation

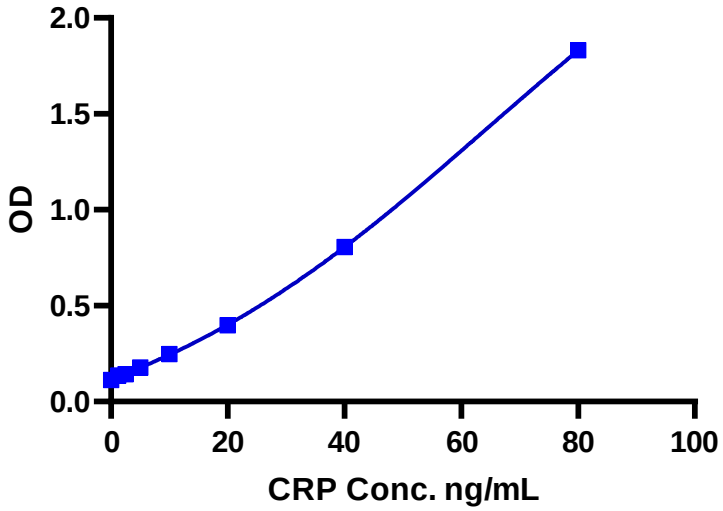
Assay Procedure

1. Add 50 µL of CRP Conjugate to each well using a repeater pipette.
2. Pipette 10 µL of either samples or standards into duplicate wells in the plate.
3. Pipette 10 µL of Assay Buffer into duplicate wells of the Zero standard.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
5. Cover the plate with the plate sealer and shake for 2 hours at room temperature. If the plate is not shaken, signals will be approximately 20 % lower.
6. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
7. Tap the plate on clean absorbent towels to dry.
8. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
9. Incubate at room temperature for 30 minutes without shaking.
10. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
11. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the Non-Specific Binding (NSB) from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in a competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The obtained concentrations should be multiplied by the dilution factor to acquire sample values.

Conversion Factor: 1.02 µg of human CRP is equivalent to 1.0 milli-International Unit



A typical standard curve of ZellX® CRP ELISA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® CRP ELISA assay was determined as 0.932 ng/mL.

Sensitivity

The sensitivity of the ZellX® CRP ELISA assay was determined as 0.616 ng/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 30 different assays over multiple days.

Item	*% CV*
Intra assay	7.0, 8.0, 7.8
Inter assay	9.9, 11.5, 9.7

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50 % binding point.

Steroid	*Cross Reactivity (%)*
human CRP	100
recombinant mouse CRP	0.0
recombinant rat CRP	0.0
recombinant porcine CRP	0.0
recombinant Pentraxin 2/SAP	0.0

Protocol summary

Add 50 µL CRP Conjugate into each well

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Add 10 µL samples/standard into duplicate wells

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Add 10 µL Assay Buffer into duplicate wells as zero

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Tap the side of the plate, mix well, Incubate 2 hours at RT with shaking

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Aspirate the plate & wash all wells 4 times with 300 µL wash buffer

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Add 100 µL of the TMB Substrate to each well

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Incubate 30 min at RT

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Add 50 µL Stop Solution to each well

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Read the absorbance at 450 nm

References

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