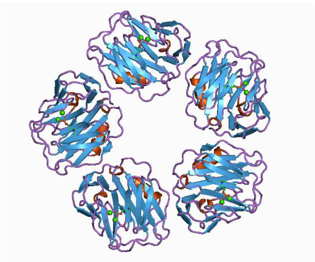
Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

High Sensitivity Human C-Reactive Protein (hsCRP)



Human C-reactive protein (CRP) is one of the so called acute phase proteins. Its concentration in blood increases rapidly as a response to inflammation. CRP is a 224 residue protein with a monomer molecular mass of approximately 25 kDa and pI 6.4 (1-4). It belongs to pentraxins, an evolutionally conserved family of proteins characterized by calcium dependent ligand binding and radial symmetry of five monomers forming a ring around central pore (5).

The precise function of CRP in vivo is still not yet completely clear. CRP has been shown to participate in inflammatory as well as innate immunity processes. Important bioactivities of CRP are determined by its ability to bind to a variety of ligands, such as damaged cell membranes, apoptotic cells and fibronectin, with the highest affinity to phosphocholine residues. When CRP is ligand-bound, it can be recognized by the complement component C1q, which leads to activation of the classical complement pathway. On the other hand, via interaction with the complement factor H, CRP regulates the alternative complement pathway (6).

CRP in diagnostics

C-reactive protein is accepted in clinical use as a major, although rather non-specific, marker of inflammation. In generally healthy people, CRP levels are usually less than 5 mg/L. In pathology, CRP concentration has an enormous, 10,000-fold dynamic range (approximately 0.05–500 mg/L) (7). The highest levels of CRP (above 30 mg/L) are observed in bacterial infection, such as septic arthritis, meningitis and pneumonia.

In 2003, the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) issued a statement that identified CRP as the inflammatory marker best suited for use in current clinical practice to assess cardiovascular risk (8). Many epidemiologic studies have indicated that CRP is a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death without known cardiovascular disease (as reviewed by Clearfield (9)). The CDC/ AHA guidelines support the use of CRP in primary prevention and set cutoff points according to relative risk categories: low risk (<1.0 mg/L), average risk (1.0-3.0 mg/L), and high risk (>3.0 mg/L). This is why present day high sensitivity CRP (hsCRP) assays are aimed at nanogram per milliliter (ng/ml) CRP level distinction.

Reagents for hsCRP assay development

These monoclonal antibodies have been used in novel immunometric assays that achieve excellent sensitivity with linear detection range from 0.025 mg/L to 2.5 mg/L in a magnetic biosensor assay (10) and from 0.01 mg/L to 50 mg/L in an immunochemiluminometric assay (11). In both assays, the detection limit was 0.004 mg/L. A detection limit of 0.0011 mg/L was reached in a solid-phase sandwich fluorescence immunoassay using nanocrystals (12). Best pairs C2-C6 and C5-CRP135 and several others provide 10,000-fold linearity in experimental immunofluorometric assays.

CLINICAL UTILITY

- **Strong, independent inflammatory marker to predict cardiovascular risk without known cardiovascular disease.**

Antibodies can be used for the development of hsCRP assays for different diagnostic platforms. In addition to monoclonal antibodies, we also provide purified native CRP and CRP-free serum.

Anti-CRP monoclonal antibodies

Applications

In native CRP molecule each protomer has two coordinated Ca^{2+} ions (13). Advanced ImmunoChemical offers anti-CRP MAbs which are either sensitive or insensitive to the absence of Ca^{2+} in the solution. Some of our antibodies recognize antigen only in the presence of Ca2+ (MAbs C3, C4). The majority of our MAbs do not depend on Ca^{2+} presence in sandwich immunoassay and are able to efficiently recognize antigen even in the presence of EDTA in the tested sample (MAbs C1, C2, C5, C6, C7, CRP11, CRP30, CRP36, CRP103, CRP135, CRP169).

Direct ELISA

All Advanced ImmunoChemical anti-CRP MAbs were tested in direct ELISA and all of them recognize native CRP with high sensitivity.

Most of the antibodies recognize nCRP both in the presence and absence of Ca^{2+}, while MAb C3 binds to CRP only in Ca^{2+} presence (Fig. 1 and Fig. 2).

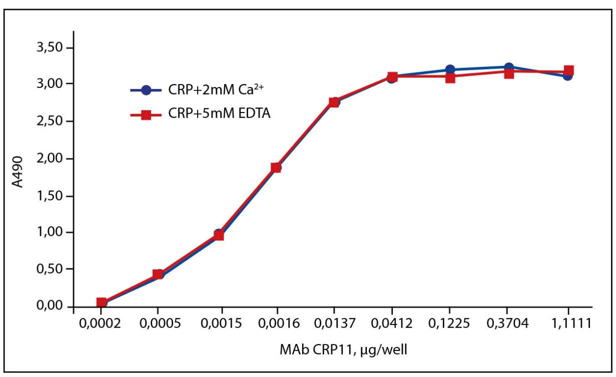
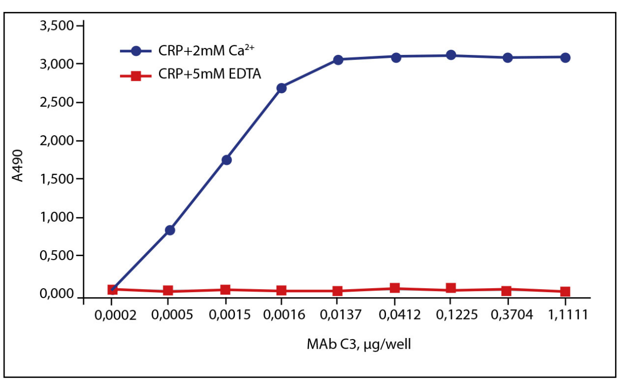


Figure 1. Interaction of MAb CRP11 with human native CRP in direct ELISA. *100 ng of native CRP (Advanced ImmunoChemical) per well was coated onto wells in Tris-buffered saline, containing 2 mM CaCl2 or 5 mM EDTA.*



CRP immunodetection in Western blotting

MAbs C1, CRP11, CRP36 and CRP169 recognize human CRP in Western blotting after antigen transfer onto nitrocellulose membrane. Results of experiments illustrating CRP immunodetection in Western blotting by MAbs CRP36 and CRP169 are presented in Fig. 3.

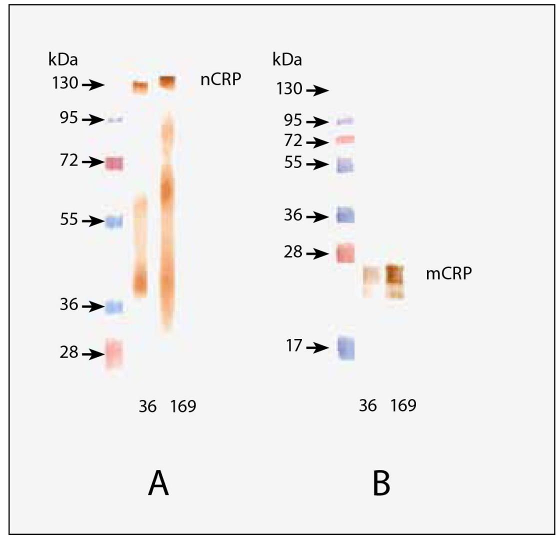


Figure 3. Immunodetection of C-reactive protein using anti-CRP MAbs in Western blotting after SDS gel electrophoresis. *Native CRP was loaded onto gel in non-reducing (A) or reducing (B) conditions. After electrophoresis protein was transferred from the gel onto nitrocellulose membrane and probed with MAbs CRP36 and CRP169.*
A: CRP in non-reducing conditions after SDS gel electrophoresis according to Taylor and van der Berg (14).
B: CRP after SDS gel electrophoresis in reducing conditions.
For visualization of MAb-CRP complex anti-mouse IgG conjugated with HRP and 3,3-Diaminobenzidine tetrahydrochloride (DAB) as HRP substrate were used.

High sensitivity CRP sandwich immunoassay

All MAbs were tested in sandwich fluoroimmunoassay as capture and detection antibodies with normal human serum (NHS) in the presence and absence of Ca^{2+} ions. The best pairs recommended for use are outlined as (capture - detection):

C2 – C6
C5 – C6
C7 – C6
C5 – CRP135
CRP30 – CRP135
C3 – C6 (Ca2+-sensitive assay)
C2 – C4 (Ca2+- sensitive assay)

The representative calibration curves for pairs C2-C6 and C5-CRP135 are shown on Fig. 4 and Fig. 5, respectively. MAbs recognize CRP antigen with excellent sensitivity and good kinetics; the linearity range is over four orders of magnitude.

Figure 2. Interaction of MAb C3 with human native CRP in direct ELISA. *100 ng of native CRP (Advanced ImmunoChemical) per well was coated onto wells in Tris- buffered saline, containing 2 mM CaCl2 or 5 mM EDTA.*

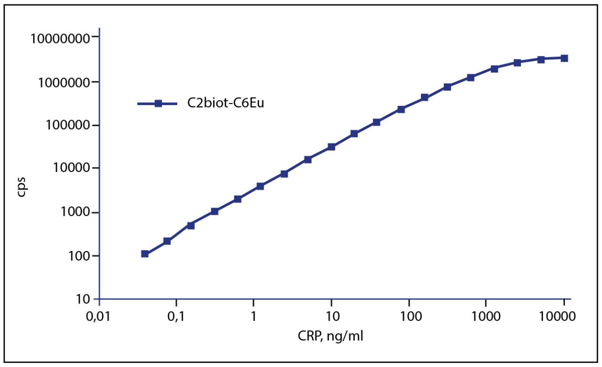


Figure 4. Immunodetection of CRP standard in sandwich immunoassay by MAb pair C2-C6.
MAb C2 is biotinylated,
MAb C6 is labeled with stable Eu3+ chelate.
Mixture of antibodies and antigen samples (100 µl) was incubated for 10 min at room temperature in streptavidin coated plates.

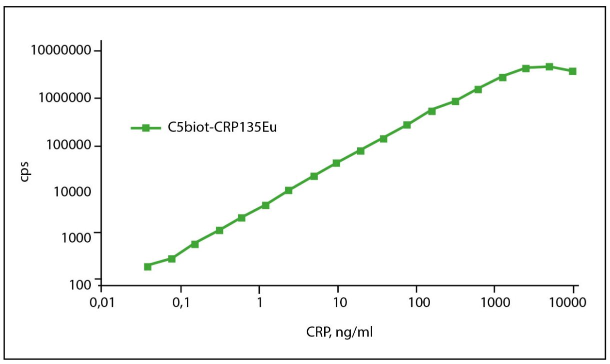


Figure 5. Immunodetection of CRP in sandwich immunoassay by *MAb pair C5-CRP135.*
MAb C5 is biotinylated,
MAb CRP135 is labeled with stable Eu3+ chelate.
Mixture of antibodies and antigen samples (100 µl) was incubated for 30 min at room temperature in streptavidin coated plates.

Several of MAbs pair recommendations are sensitive to the presence of EDTA in solution, whereas others are not affected by this presence (Fig. 6). The pair C5-CRP135 as well as some others could be used both in the presence and absence of Ca2+ ions. The C3-C6 MAbs combination is strongly calcium-dependent.

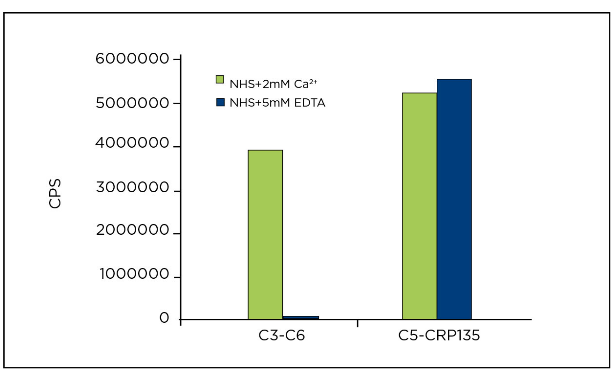


Figure 6. Influence of EDTA on CRP measurements. *Two different mAb pairs were used in a sandwich immunoassay. Pair C3-C6 (left) shows a dependence on Ca2+ as it fails to recognize CRP in the presence of EDTA. In contrast, pair C5-CRP135 (right) is unaffected by EDTA in the solution. Normal human serum supplemented with 2 mM CaCl2 or 5 mM EDTA was used as the source of CRP.*

Affinity information

For some applications, such as turbidimetry, nephelometry and competitive immunoassay, affinity constants of utilized antibodies have to be estimated. Advanced ImmunoChemical offers a panel of MAbs with different affinity constants (Biacore®). Biacore methodology is based on the surface-enhanced plasmon resonance effect. It enables the assessment of interaction between two partners in real-time. Rate constants of associations and dissociation can be visualized and affinity constant can be derived.

MAb	Kd (M)
C2	1.93×10^{-9}
C5	1.7×10^{-8}
CRP30	4.3×10^{-8}
CRP103	5.2×10^{-8}
CRP135	4.4×10^{-9}

Table 1. Affinity constants of selected anti-CRP MAbs.

CRP free serum

CRP free serum is prepared from pooled normal human serum by immunoaffinity chromatography. The matrix for affinity sorbent utilizes three monoclonal antibodies each with a different epitope specificity. According to sandwich immunoassay testing the CRP free serum contains less than 0.02 µg/ml of human CRP (Fig. 8). CRP free serum can be used as a matrix for standard and calibrator preparations.

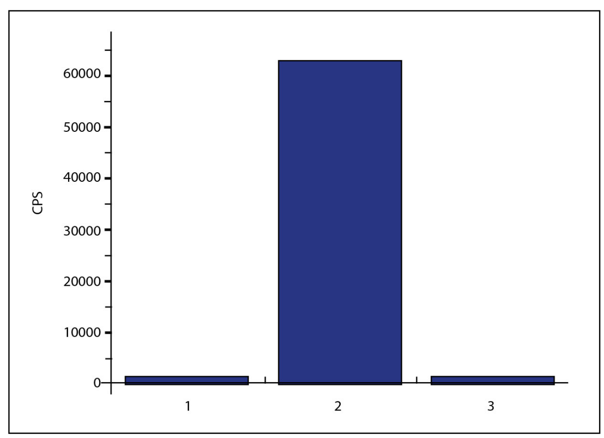


Figure 8. CRP level in normal human serum and in CRP free serum detected in sandwich-immunoassay.
1. Buffer
2. Initial signal in normal human serum 25 fold diluted (corresponds to 4 µg/ml of CRP)
3. Residual signal in CRP free serum

Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
C-reactive protein	2-CRP	C1	IgG2b	EIA, WB, high sensitivity
		C2	IgG1	EIA, high sensitivity
		C3	IgG1	EIA, IHC, CA^{2+} dependent, high sensitivity
		C4	IgG1	EIA, CA^{2+} dependent, high sensitivity
		C5	IgG2a	EIA, high sensitivity
		C6	IgG1	EIA, high sensitivity
		C7	IgG1	EIA, IHC, high sensitivity
		CRP11	IgG1	EIA, WB
		CRP30	IgG1	EIA, low affinity
		CRP36	IgG2a	EIA, WB, IHC
		CRP103	IgG2b	EIA, low affinity
		CRP135	IgG2b	EIA, high sensitivity
		CRP169	IgG2a	EIA, WB
C-reactive protein, *in vitro*	2-CRPiv	C6cc	IgG2a	EIA, high sensitivity
		C2cc	IgG1	EIA, WB

ANTIGENS

Product Name	Cat. #	Purity	Source
Human C-reactive protein	8-CRP	>95%	Human pleural, ascitic fluid or plasma

DEPLETED SERUM

Product Name	Cat. #	Source
C-reactive protein free serum	11-CRP-fs	Pooled normal human serum

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