Human C-Reactive Protein (CRP)

Human C-reactive protein (CRP) is one of the so-called acute phase proteins. CRP is produced in liver and its concentration in blood increases rapidly as a response to inflammation. It is routinely used as a non-specific marker of 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 total mass of the CRP pentamer is approximately 120 kDa.

The precise function of CRP *in vivo* is still not yet completely clear, but it has been shown that CRP increases by the secretion of interleukin-6 produced by for example macrophages and T cells (6). CRP has been shown to participate in inflammatory as well as innate immunity processes. The level of CRP in the blood of healthy people is usually low but in infections caused by bacteria the concentration of CRP can quite easily increase tenfold. In contrast, infections of viral origin usually result in just a moderate increase in the level of CRP. 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 (7).

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) (8). The highest levels of CRP (above 30 mg/L) are observed in bacterial infection, such as septic arthritis, meningitis, and pneumonia.

CLINICAL UTILITY

- **Prediction of future cardiovascular risk**
- **Inflammation**

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 (9). 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 (10)). 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, whereas traditional CRP measurement detects the range of 10 to 1,000 mg/L (11).

The elevated CRP-levels can be seen in diseases described in Table 1. The routine clinical use of CRP measurement includes for example screening test for organic diseases, assessment of disease activity in inflammatory conditions, diagnosis and management of infections, and differential diagnosis or classification of inflammatory disease (12).

Reagents for hsCRP assay development

Hytest's 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 (13) and from 0.01 mg/L to 50 mg/L in an immunochemiluminometric assay (14). 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 (15). Our best pairs C2cc-C6cc and C5-CRP135cc and several others provide 10,000-fold linearity in experimental immunofluorometric assays. Our antibodies could be used for the development of hsCRP assays for different diagnostic platforms. In addition to monoclonal antibodies, we also provide recombinant human CRP and CRP-free serum.

Table 1.
CRP responses in disease. Major acute-phase CRP response.

Infections	Bacterial Systemic/Severe fungal
Allergic complications of infection	Rheumatic fever Erythema nodosum
Inflammatory disease	Rheumatoid arthritis Juvenile chronic arthritis Ankylosing spondylitis Psoriatic arthritis Systemic vasculitis Polymyalgia rheumatica Reiter disease Crohn disease Familial Mediterranean fever
Necrosis	Myocardial infarction Tumor embolization Acute pancreatitis
Trauma	Surgery Burns Fractures
Malignancy	Lymphoma Carcinoma Sarcoma

MONOCLONAL ANTIBODIES SPECIFIC TO CRP

Applications

In native CRP molecule each protomer has two coordinated Ca^{2+} ions (16). Hytest 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 Ca^{2+} (MAbs C3, C4cc). The majority of Hytest 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, C2cc, C5, C6cc, C7, CRP11, CRP30cc, CRP36, CRP135cc, CRP169).

All Hytest anti-CRP MAbs were tested in different immunological applications.

Direct ELISA

All Hytest anti-CRP MAbs were tested in direct ELISA and all of them recognize native CRP with high sensitivity. Most of the antibodies recognize native CRP 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).

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.

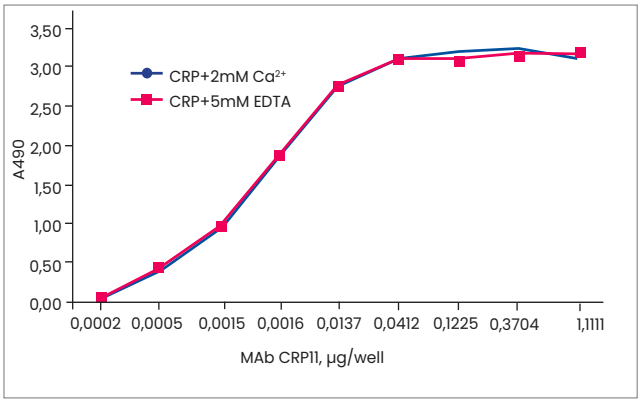

Figure 1.
Interaction of MAb CRP11 with human native CRP in direct ELISA. 100 ng of native CRP per well was coated onto wells in Tris-buffered saline, containing 2 mM $CaCl_2$ or 5 mM EDTA.

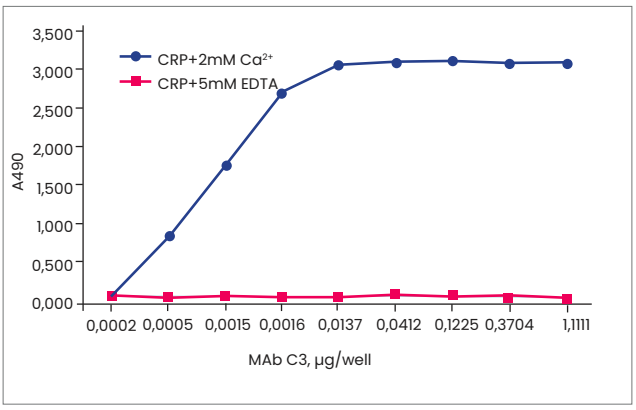

Figure 2.
Interaction of MAb C3 with human native CRP in direct ELISA. 100 ng of native CRP per well was coated onto wells in Tris-buffered saline, containing 2 mM $CaCl_2$ or 5 mM EDTA.

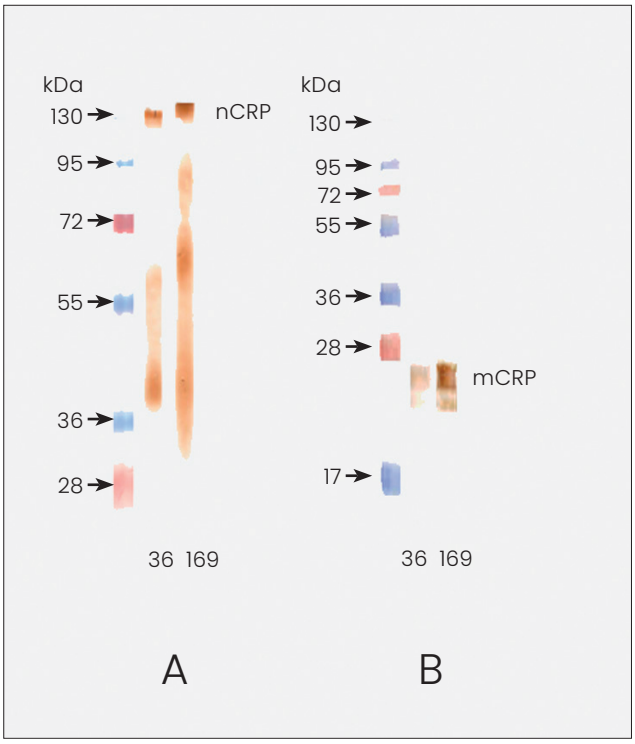

Figure 3.
Immunodetection of C-reactive protein using anti-CRP MAbs in Western blotting after SDS gel electrophoresis. Native CRP was loaded onto gel in non-reducing (A) or reducing (B) conditions. After electrophoresis protein was transferred from the gel onto nitrocellulose membrane and probed with 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):

C2cc – C6cc
C5 – C6cc
C7 – C6cc
C5 – CRP135cc
CRP30cc – CRP135cc
C3 – C6cc (Ca^{2+}-sensitive assay)
C2cc – C4cc (Ca^{2+}- sensitive assay)

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

Several of Hytest's MAb pair recommendations are sensitive to the presence of EDTA in the 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 Ca^{2+} ions. The C3-C6 MAbs combination is strongly calcium-dependent.

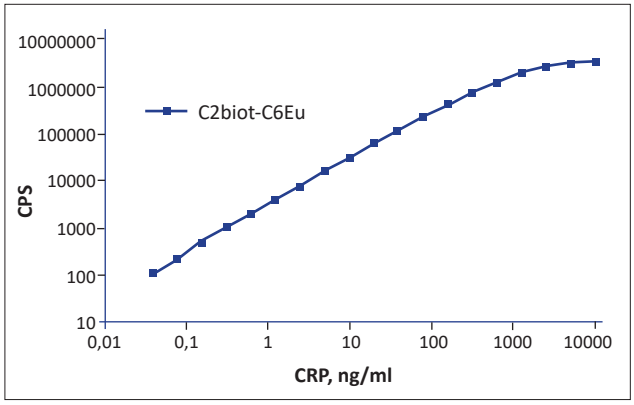

Figure 4.
Immunodetection of CRP standard in sandwich immunoassay by MAb pair C2-C6.
MAb C2 is biotinylated,
MAb C6 is labeled with stable Eu^{3+} chelate.
Mixture of antibodies and antigen samples (100 µl) was incubated for 10 min at room temperature in streptavidin coated plates.

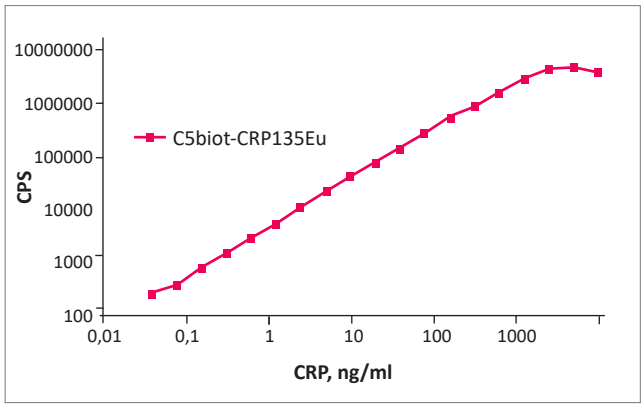

Figure 5.
Immunodetection of CRP in sandwich immunoassay by MAb pair C5-CRP135.
MAb C5 is biotinylated,
MAb CRP135 is labeled with stable Eu^{3+} chelate.
Mixture of antibodies and antigen samples (100 µl) was incubated for 30 min at room temperature in streptavidin coated plates.

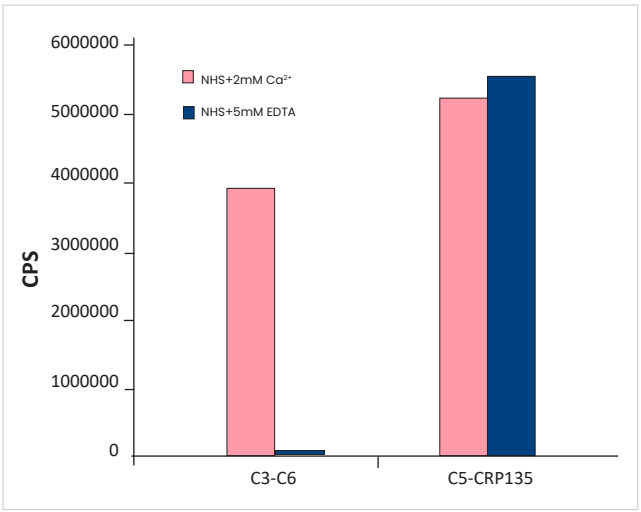

Figure 6.
Influence of EDTA on CRP measurements. Two different MAb pairs were used in a sandwich immunoassay. Pair C3-C6 (left) shows a dependence on Ca^{2+} as it fails to recognize CRP in the presence of EDTA. In contrast, pair C5-CRP135 (right) is unaffected by EDTA in the solution. Normal human serum supplemented with 2 mM $CaCl_2$ 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 needs to be estimated. Hytest offers a panel of MAbs with different affinity (Table 2) and we have estimated affinity constants for some of Hytest anti-CRP MAbs by using the Biacore® technique. 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 could be visualized, and affinity constant could therefore be derived. The kinetics of our selected monoclonal antibodies varies which makes them suitable for wide dynamic range CRP immunoassay development. The selected kinetic values of our anti-CRP antibodies are visualized in Figure 7.

Table 2.
Affinity constants of selected Hytest anti-CRP MAbs.

MAb	Kon (1/Ms)	Koff (1/s)	Kd (M)
C2cc	2.3 x 10^{5}	4.4 x 10^{-4}	1.93×10^{-9}
C5	1.3 x 10^{5}	2.2 x 10^{-3}	1.7×10^{-8}
CRP30cc	9.3 x 10^{3}	4.0 x 10^{-3}	4.3×10^{-7}
CRP135cc	1.5 x 10^{5}	6.6 x 10^{-4}	4.4×10^{-9}

Figure 7.
Kinetic values of selected Hytest anti-CRP MAbs. The on-rate values are shown on the left side axis and off-rate values are shown on the right side axis for selected Hytest anti-CRP MAbs (Cat.# 4C28/4C28cc).

RECOMBINANT HUMAN CRP

De novo hepatic synthesis of CRP starts rapidly after a single systemic stimulus and the serum concentrations rise above 5 mg/mL in approximately 6 hours and reaches a peak in about 48 hours. In plasma the half-life of CRP is about 19 hours and is constant under all health and disease conditions (12).

Therefore, the concentration of circulating CRP is solely dependent on the synthesis rate, which respectively directly reflects the intensity of the pathological processes stimulating the CRP production (17).

Hytest recombinant human CRP is expressed in mammalian cells and purified in native conditions that excludes renaturation steps. Recombinant human CRP is purified by affinity chromatography with phosphatidylcholine matrix that confirms functional activity of the recombinant protein. Our recombinant human CRP (Cat.# 8CR8) does not contain any tags. The protein presentation is optimized for storage in liquid state. The purity of the protein is >95%, Fig. 8.

Recombinant human CRP is immunochemically active in different sandwich immunoassay pairs using Hytest antibodies, Fig. 9.

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 with different epitope specificity. According to internal sandwich immunoassay testing the CRP free serum contains less than 0.02 µg/ml of human CRP (Fig. 10). CRP free serum can be used as a matrix for standard and calibrator preparations.

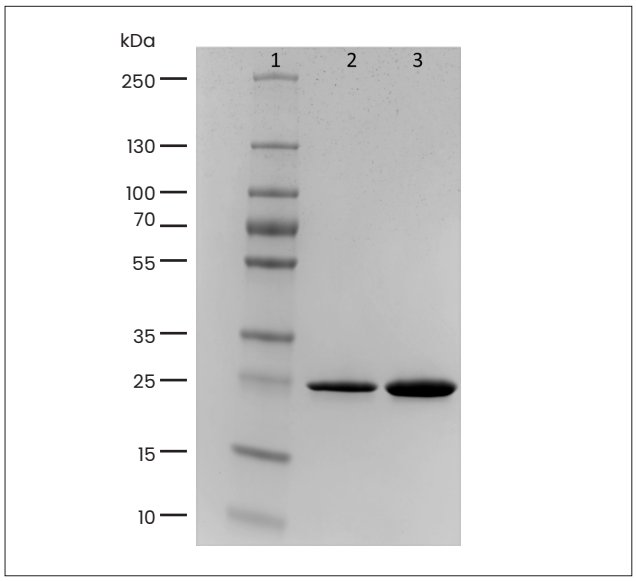

Figure 8.
SDS-PAGE of recombinant human CRP in reducing conditions.
1) molecular weight markers
2) recombinant human CRP, 2 µg
3) recombinant human CRP, 5 µg

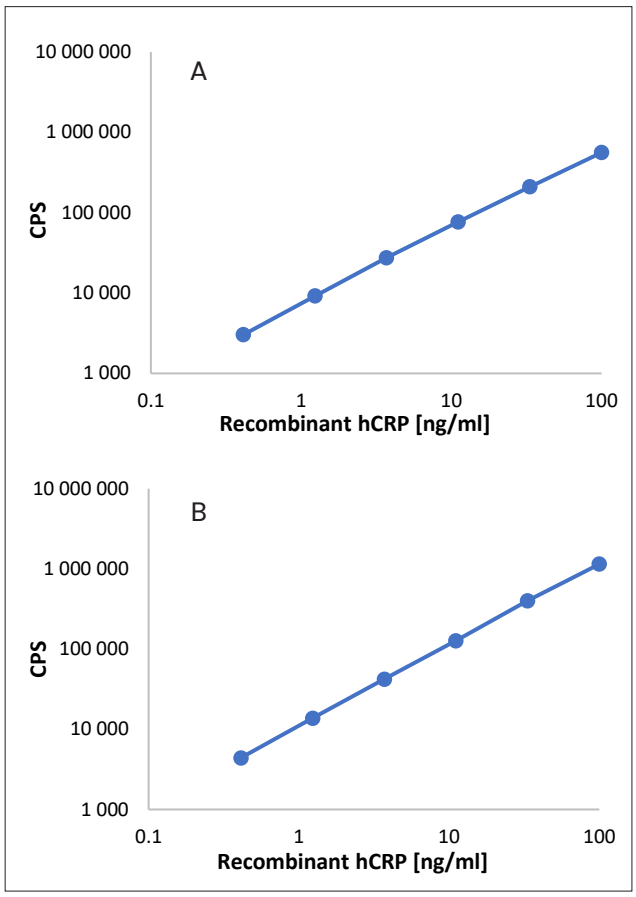

Figure 9.
Calibration curve for the recombinant human CRP. A) Calibration curve obtained with CRP30cc-CRP135cc and B) with C2cc-C4cc (capture-detection) sandwich immunoassays. The capture antibodies were adsorbed on the immunoassay microplates. A mixture of antigen and detection antibodies labelled with Eu^{3+} stable chelate was incubated for 30 minutes at room temperature.

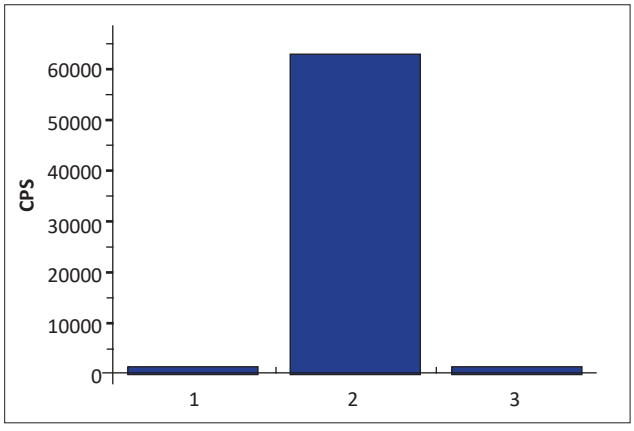

Figure 10.
CRP level in normal human serum and in CRP free serum detected in sandwich-immunoassay. 1. Buffer, 2. Initial signal in normal human serum 25 fold diluted (corresponds to 4 µg/ml of CRP), 3. Residual signal in CRP free serum.

REFERENCES

1. **Yasojima Koji et al.** " Generation of C-Reactive Protein and Complement Components in Atherosclerotic Plaques." Am J Pathol. 2001 March; 158(3): 1039–1051.
2. **Kobayashi S, Inoue N, et al.** "Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein." Arterioscler Thromb Vasc Biol 2003, 23:1398–1404.
3. **Ciubotaru I., Potempa L.A., Wander R.C.** "Production of Modified C-Reactive Proteinin U937-Derived Macrophages". Exp Biol Med (Maywood) 2005, 230(10):762-70.
4. **Diehl E. E. et al.** "Immunohistochemical Localization of modified C-reactive protein antigen in normal vascular tissue." American Journal of the Medical Sciences 2000; 319(2):79.
5. **Hirschfield G.M., Pepys M.B.** "C-reactive protein and cardiovascular disease: new insights from an old molecule." Q J Med 2003; 96:793-807.
6. **Thompson D., Pepys M.B., Wood S.P.** "The physiological structure of human Creactive protein and its complex with phosphocholine." Structure 1999; 7:169-177.
7. **Bıro A. et al.** "Studies on the interactions between C-reactive protein and complement proteins" Immunology 2007 May;121(1):40-50.
8. **Lowe G.D.O., Pepys M.B.** "C-Reactive Protein and Cardiovascular Disease: Weighing the Evidence" Current Atherosclerosis Reports 2006, 8:421–428.
9. **Ridker P.M.** "C-reactive protein: a simple test to help predict risk of heart attack and stroke" Circulation. 2003; 108:e81-e85.
10. **Clearfield M.B.** "C-reactive protein: a new risk assessment tool for cardiovascular disease" JAOA 2005; 105(9):409-416.
11. **Pearson T.A et al.** "Markers of inflammation and cardiovascular disease: application and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association". Circulation 2003 Jan 28;107(3):499-511.
12. **Pepys M.B & Hirschfield G.M.** "C-reactive protein: a critical update." J Clin Invest Jun 2003;111(12):1805-1812.
13. **Meyer M.H. et al.** "CRP determination based on a novel magnetic biosensor."Biosens Bioelectron 2007 Jan 15; 22(6):973-9.
14. **Shiesh S.C. et al.** "Determination of C-reactive protein with an ultra-sensitivity immunochemiluminometric assay". J Immunol Methods 2006 Apr 20;311(1-2):87-95.
15. **Sin K.K. et al.** "Fluorogenic nanocrystals for highly sensitive detection of C-reactive protein." IEE Proc Nanobiotechnol 2006 Jun;153(3):54-8.
16. **Karolina E. Taylor and Carmen W. van den Berg** "Structural and functional comparison of native pentameric, denatured monomeric and biotinylated C-reactive protein." Immunology 2006; 120, 404–411.
17. **Gershov D. et al.** "C-reactive protein binds to apoptic cells, protects the cells from assembly of the terminal complement components, and sustains an anti-inflammatory innate immune response: implications for systemic autoimmunity." J Exp Med 2000;192:1353-1363.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
C-reactive protein	4C28cc	C2cc	IgG1	*In vitro*, EIA, high sensitivity
		C4cc	IgG1	*In vitro*, EIA, Ca^{2+} dependent, high sensitivity
		C6cc	IgG2a	*In vitro*, EIA, high sensitivity
		CRP30cc	IgG1	*In vitro*, EIA, low affinity
		CRP135cc	IgG2b	*In vitro*, EIA, high sensitivity
	4C28	C1	IgG2b	EIA, WB , high sensitivity
		C3	IgG1	EIA, IHC, Ca^{2+} dependent, high sensitivity
		C5	IgG1	EIA, high sensitivity
		C7	IgG1	EIA, IHC, high sensitivity
		CRP11	IgG1	EIA, WB
		CRP36	IgG2a	EIA, WB, IHC
		CRP169	IgG2a	EIA, WB

ANTIGEN

Product name	Cat. #	Purity	Source
C-reactive protein (CRP), human, recombinant	8CR8	>95%	Recombinant

DEPLETED SERUM

Product name	Cat. #	Source
C-reactive protein free serum	8CFS	Pooled normal human serum

Please note that some or all data presented in this Technotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**