HyTest

Inflammation

Antibodies and antigens

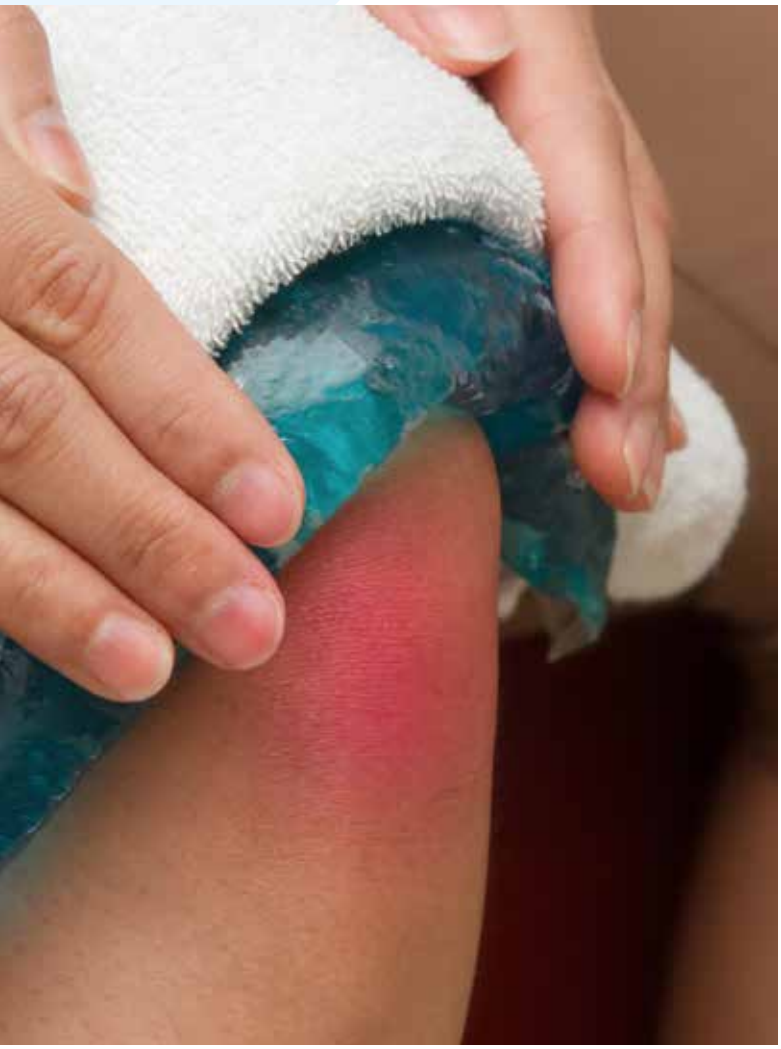



Introduction

Inflammation is body's response to physical, biological or chemical irritants. These include tissue injuries, burns and infections by microbes but also pathological situations that lead to atherosclerosis or ischemia, for example. The inflammatory reaction is protective and tightly regulated. Its purpose is to eliminate the cause of inflammation, get rid of dead cells and to initiate tissue repair. Inflammation can be both acute and chronic. Acute inflammation occurs instantly as a response to inflammation stimuli and the signs are often prominent like redness of the skin or swelling. In contrast, the onset of chronic inflammation is slower and the local signs may be much more subtle which makes the diagnosis more difficult.

We provide immunological reagents — antibodies and antigens — that allow development of quantitative immunoassays for detecting various inflammatory markers. These include C-reactive protein which is a non-specific marker of inflammation, procalcitonin which is useful when diagnosing sepsis and a selection of inflammatory mediators like interleukins and interferons.

Note that in this brochure the monoclonal antibodies (mAbs) are listed only according to the analyte they recognize. In most cases there are several different mAbs available under one catalogue number. More detailed information regarding the performance of our products, full list of individual mAbs and recommendations for capture-detection antibody pairs (when available) can be found on our website — www.hytest.fi. You are also most welcome to contact our Tech Support Team directly by writing to support@hytest.fi.



Procalcitonin (PCT)

CLINICAL UTILITY

- **Systemic inflammation**
- **Sepsis**

Procalcitonin (PCT) is considered to be the main marker of disorders that are accompanied by systemic inflammation and sepsis. The association between the elevated levels of PCT in blood with systemic inflammation has been known since the 1990s.

PCT is a good marker of bacterial infection as its basal level in blood is very low and viral infections only cause a slight increase in its concentration. In addition, the concentration of PCT closely correlates with the severity of inflammation, which further supports the diagnostic value of the marker.

Detecting PCT in human serum

PCT is a 116 amino acid prohormone that can be processed into three fragments: N-terminal PCT, calcitonin and katacalcin (Figure 1). While in normal conditions the amount of non-cleaved PCT in blood is low, it increases during systemic inflammation and sepsis.

We have developed several monoclonal antibodies specific to different fragments of PCT. Figure 2 shows titration curves of serums from two septic patients and one healthy human. The capture and detection antibodies in this assay are specific to the calcitonin and N-terminal PCT fragments respectively. However, also other mAb combinations can be used for developing an assay to detect PCT.

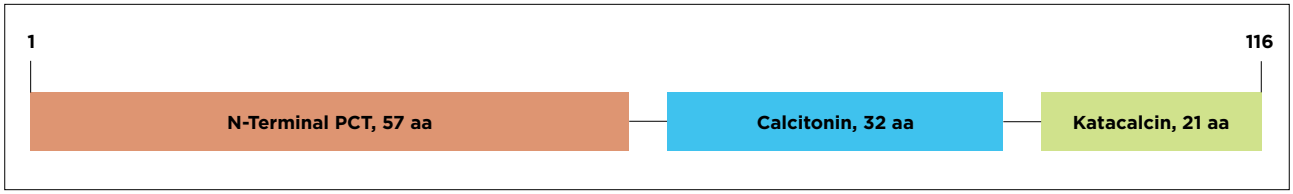


Figure 1. Processing of PCT. PCT is a small prohormone (~13 kDa) that is synthesized by the C-cells of the thyroid glands. During maturation, PCT undergoes successive cleavages to form three molecules: N-terminal PCT, calcitonin and katacalcin. In normal conditions, the hormonally active calcitonin can be found in blood whereas the basal level of PCT is very low. The "inflammatory" PCT detected in blood during systemic inflammation is not produced in C-cells but is instead produced in other tissue types.

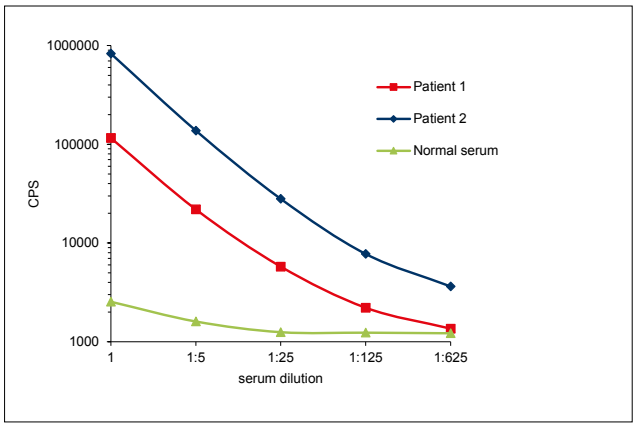


Figure 2. Titration of human serum samples. Serum from two patients with sepsis of bacterial origin and one healthy human (normal serum) were used as samples in a sandwich fluoroimmunoassay.
Capture mAb 16B5: 1 µg/well
Detection mAb 42 (Eu^{3+}-labeled): 0.1 µg/well.
Incubation time: 45 min.

Recombinant human PCT with no tag

Our recombinant human PCT is expressed in *E. coli* as a full length, 116 amino acid polypeptide without a signal peptide and with no affinity tags. The sequence corresponds to UniProt P01258 lacking a signal peptide. The purified antibody is stable and retains its activity well after repeated freeze-thaw cycles (see Figure 3). The recombinant PCT is suitable for use as a calibrator in procalcitonin or calcitonin immunoassays.

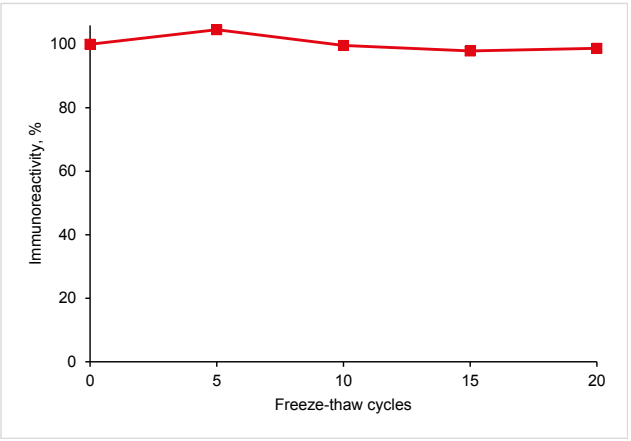


Figure 3. Stability of PCT solution after repeated freeze-thaw cycles. PCT at 1 mg/ml concentration was frozen at -70°C and thawed at room temperature for the indicated number of times. Immunoreactivity was measured in a sandwich ELISA with mAb pair 16B5-42.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4C10*	Monoclonal mouse anti-human calcitonin	Enzyme immunoassays Western blotting
4C10cc	Monoclonal mouse anti-human calcitonin, *in vitro*	Enzyme immunoassays Western blotting
4PC47*	Monoclonal mouse anti-human procalcitonin	Enzyme immunoassays Western blotting

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

POLYCLONAL ANTIBODY

Cat.#	Product	Host	Tested applications
PPC3	Polyclonal anti-procalcitonin	Goat	Enzyme immunoassays

ANTIGEN

Cat.#	Product	Source	Purity
8PC5	Procalcitonin, tag-free, recombinant	Recombinant	> 95%

C-reactive protein (CRP)

CLINICAL UTILITY

- **Non-specific marker of inflammation and infection**
- **Prediction of future cardiovascular risk**

C-reactive protein (CRP) is produced by the liver and is one of the so-called acute phase proteins. It is routinely used as a non-specific marker of inflammation. Its concentration in blood increases rapidly and considerably as a response to inflammation or infection. The level of CRP in the blood of healthy people is usually less than 10 mg/L. 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.

CRP binds to damaged cell membranes, apoptotic cells and bacteria. It has a high affinity towards phosphocholine but has also been shown to bind to other ligands. While the CRP-ligand complex can activate the classical complement pathway, the precise function of CRP *in vivo* is still not yet completely clear.

Human CRP found in blood is a non-glycosylated pentamer formed from five identical subunits that are non-covalently bound to each other (Figure 4). Each subunit has a Ca^{2+}-dependent ligand binding site. CRP is a member of a family called pentraxins.

Effect of Ca^{2+} on immunodetection of CRP using HyTest anti-CRP mAbs

We offer over ten different monoclonal antibodies specific to human CRP. The majority of these mAbs are unaffected by the presence or absence of Ca^{2+} in the quantitative detection of CRP. However, some antibodies and antibody pairs depend on Ca^{2+} and the binding of CRP may be abolished in the presence of EDTA. This should be kept in mind when designing a CRP immunoassay. Figure 5 shows an example of two antibody pairs — one that is dependent and another that is independent of the presence of Ca^{2+}.

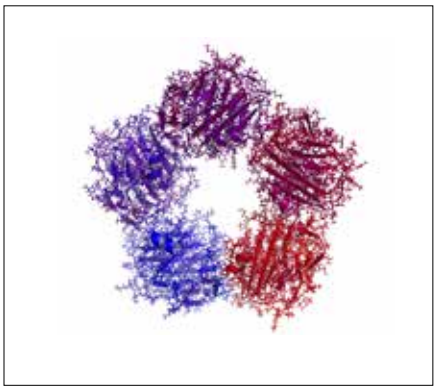


Figure 4. CRP is a pentamer that is composed of five identical subunits that form a ring like structure.

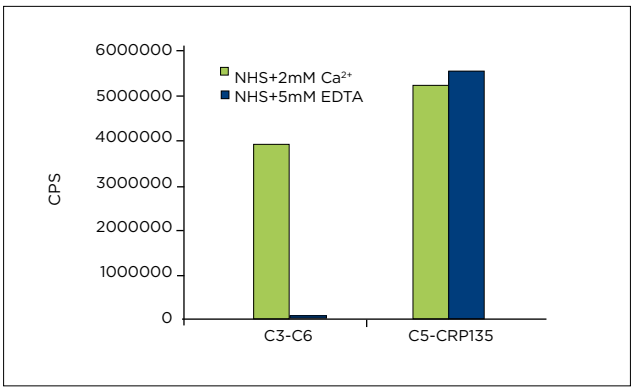


Figure 5. Influence of EDTA on CRP measurements. Two different mAb pairs were used in a sandwich immunoassay. Pair C3-C6 (left) shows a dependence on Ca^{2+} as it fails to recognize CRP in the presence of EDTA. In contrast, pair C5-CRP135 (right) is unaffected by EDTA in the solution. Normal human serum supplemented with 2 mM $CaCl_2$ or 5 mM EDTA was used as the source of CRP.

Antibodies of different affinity

Anti-CRP antibodies developed by HyTest have been utilized in several immunoassays and achieved excellent sensitivity and a broad linear detection range (Meyer et al., 2007; Shiesh et al., 2006; Sin et al., 2006).

These antibody combinations could be used for the development of CRP assays for different diagnostic platforms. For the convenience of our customers, we have monoclonal antibodies with different affinities (Figure 6 and Table 1), which therefore enable them to be used in different types of immunoassays.

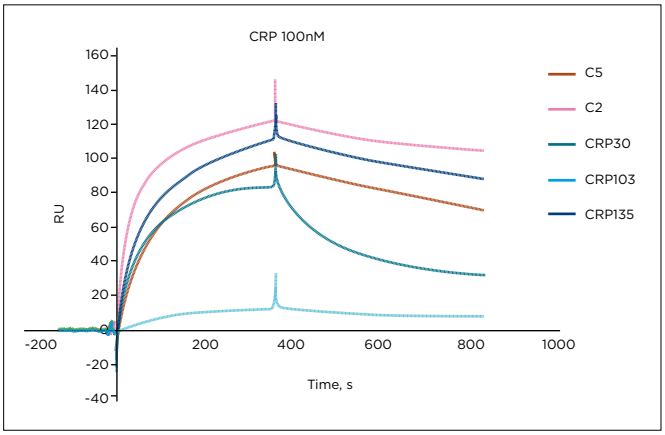


Figure 6. Biacore X sensograms of five different anti-CRP mAbs. 100 nM native CRP was exposed to the chip-immobilized mAbs in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate 20, pH 7.4).

Table 1. Affinity constants for selected anti-CRP mAbs.

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}

High-sensitivity CRP (hsCRP)

It should be noted that CRP is also used as a marker of increased risk for cardiac diseases. In this, it is the basal level of CRP that has more clinical significance and therefore, the assays need to be highly sensitive and aimed at nanogram per milliliter CRP level distinction.

Our anti-CRP antibodies are suitable for the development of a quantitative hsCRP assay. For more information, please see the hsCRP TechNotes available on our website www.hytest.fi.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4C28*	Monoclonal mouse anti-human C-reactive protein	Enzyme immunoassays Western blotting Turbidimetric assays Immunohistochemistry Immunoaffinity purification
4C28cc	Monoclonal mouse anti-human C-reactive protein, *in vitro*	Enzyme immunoassays

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8C72	C-reactive protein	Human pleural/ascetic fluid or plasma	> 95%

DEPLETED SERUM

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

Additional products

Serum amyloid A (SAA)

Serum amyloid A (SAA) proteins form a family of apolipoproteins. In human blood they are mostly found in association with high density lipoprotein (HDL). Several SAA proteins have been identified. Some SAAs are expressed constitutively while others are expressed in response to inflammation. The latter SAAs belong to a group of acute phase proteins and their level in blood increases rapidly by up to 1000-fold after tissue injury or other inflammatory stimulus.

We provide several anti-SAA monoclonal antibodies that are suitable for the development of a quantitative SAA immunoassay.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4SA11*	Monoclonal mouse anti-serum amyloid A	Enzyme immunoassays Western blotting

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8SA1	Serum amyloid A1 (SAA1), human, recombinant	Recombinant	> 95%
8SA2	Serum amyloid A2 (SAA2), human, recombinant	Recombinant	> 95%

Tumor necrosis factor (TNF), alpha

Tumor necrosis factor alpha (TNF-α) is a cytokine that regulates how the body responds to infections. TNF-α has both growth stimulating and growth inhibitory properties that depends upon the signaling pathway on which it acts. The production of TNF-α is induced by lipopolysaccharide and other compounds that originate from microorganisms which invade the body. It is released by activated macrophages and also by other cell types such as mast cells and neurons. TNF-α is one of the acute phase proteins.

We provide several monoclonal antibodies specific to TNF-α.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4T10*	Monoclonal mouse anti-human tumor necrosis factor alpha	Enzyme immunoassays Western blotting Immunohistochemistry

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

Interferons

Interferons are a group of proteins, the expression and secretion of which is induced in the cells of the immune system as a response to the presence of viruses, bacteria or other pathogens. The proteins help the body to defend against pathogens by boosting the immune system response. Interferons belong to the class of cell signaling molecules called cytokines.

There are three major classes of interferons: type I, type II and type III. We provide several monoclonal antibodies specific to type I interferon alpha, as well as to interferon gamma, which belongs to type II interferons.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4IF13*	Monoclonal mouse anti-human interferon alpha	Enzyme immunoassays Western blotting
4I22*	Monoclonal mouse anti-human interferon gamma	Enzyme immunoassays Western blotting

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

Interleukins

Interleukins play a major role in regulating the immune system. In a similar manner to interferons, they also belong to the group of cytokines. Interleukins are mainly produced by leukocytes and also by other cell types. Interleukins influence the development, differentiation and activation of lymphocytes and therefore help the body to defend against infections.

Interleukins form a group of proteins that consist of several protein families. We provide several monoclonal antibodies specific to some of the most common interleukin proteins.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4IL12*	Monoclonal mouse anti-human interleukin-1, beta	Enzyme immunoassays Immunohistochemistry
4IL34*	Monoclonal mouse anti-human recombinant interleukin 2	Enzyme immunoassays
4IL35*	Monoclonal mouse anti-human recombinant interleukin 3	Enzyme immunoassays
4IL36	Monoclonal mouse anti-human recombinant interleukin 4	Enzyme immunoassays
4IL6*	Monoclonal mouse anti-human recombinant interleukin 6	Enzyme immunoassays
4IL17*	Monoclonal mouse anti-human recombinant interleukin 17a	Enzyme immunoassays

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

Erythropoetin

Erythropoetin is a glycoprotein that is produced by fibroblasts in the kidney as a response to the low oxygen level of blood. Erythropoetin belongs to the group of cytokines and it is essential in erythropoiesis. It mediates the production of red blood cells by acting on red blood cell precursors.

In addition to its role in erythropoiesis, erythropoetin is also involved in several other signaling pathways. For example, it negatively regulates the production of inflammatory cytokines in the central nervous system as a response to ischemic and traumatic injuries.

We provide several monoclonal antibodies specific to erythropoetin.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4ER1*	Monoclonal mouse anti-erythropoetin	Enzyme immunoassays Western blotting

*Note. Several mAbs available under one catalogue number. Please see www.hytest.fi.

Epidermal growth factor (EGF)

Epidermal growth factor (EGF) stimulates cell growth, proliferation and differentiation. It is synthesized in various cell types and can be found in saliva, milk, urine and plasma. EGF has been shown to regulate intestinal healing and it has been suggested to have an anti-inflammatory effect on intestinal inflammatory diseases.

We provide one monoclonal antibody specific to EGF.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4EL36	Monoclonal mouse anti-human epidermal growth factor	Enzyme immunoassays Western blotting

References

Meyer M.H. et al. CRP determination based on a novel magnetic biosensor. Biosens. Bioelectron. 2007, 22(6):973-979.

Shiesh S.C. et al. Determination of C-reactive protein with an ultra-sensitivity immunochemiluminometric assay. J. Immunol. Methods 2006, 311(1-2):87-95.

Sin K.K. et al. Fluorogenic nanocrystals for highly sensitive detection of C-reactive protein. IEE Proc. Nanobiotechnol. 2006, 153(3):54-58.

Together. Today and Tomorrow.

www.hytest.fi

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