



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, a 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 Sales Team directly by writing to hytest@hytest.fi.

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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.

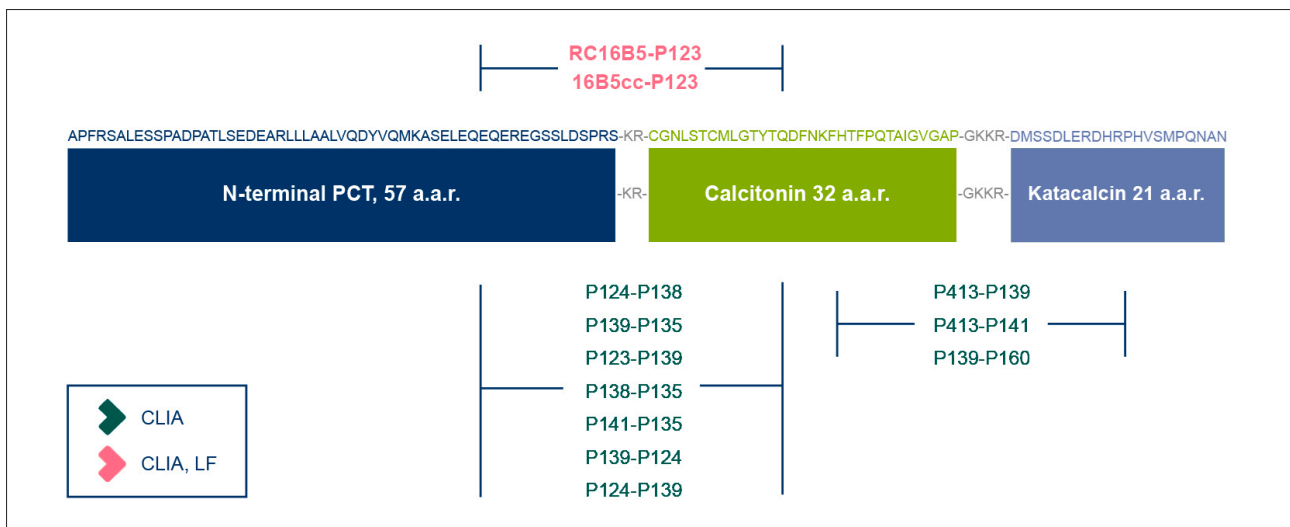


Figure 1.

The amino acid sequence of human procalcitonin (116 a.a.r.), representing N-terminal PCT (1-57), calcitonin (60-91), and katacalcin (96-116), and pairs of MAbs recommended for PCT sandwich immunoassays (capture-detection).

We also tested several assays for their ability to detect native PCT in human serum. Serum samples from two septic patients and one healthy individual were analyzed using different combinations of anti-PCT MABs. The correlations of the developed assays with the Roche Elecsys BRAHMS PCT were calculated (Figure 3). All samples were tested for PCT concentration on the Cobas 411 or the 6000 analyzers (Roche) and compared with our in-house PCT assays using the CLIA method.

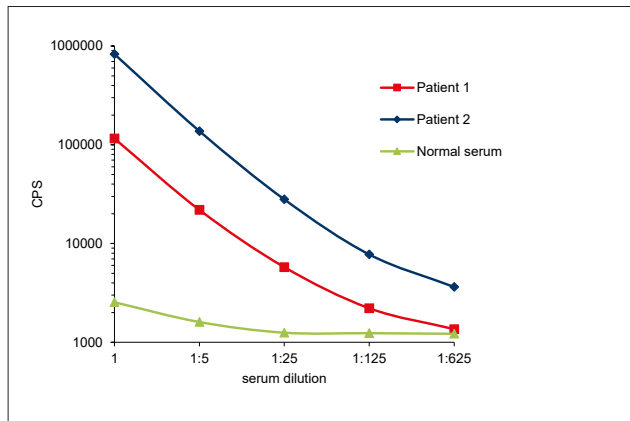


Figure 2.
Titration of human serum samples. Serum from two patients with sepsis of bacterial origin and one healthy human (normal serum) were used as samples in a sandwich fluoroimmunoassay.
 Capture MAb 16B5: 1 µg/well
 Detection MAb 42 (Eu³⁺-labeled): 0.1 µg/well.
 Incubation time: 45 min.

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. The recombinant PCT is suitable for use as a calibrator in procalcitonin or calcitonin immunoassays.

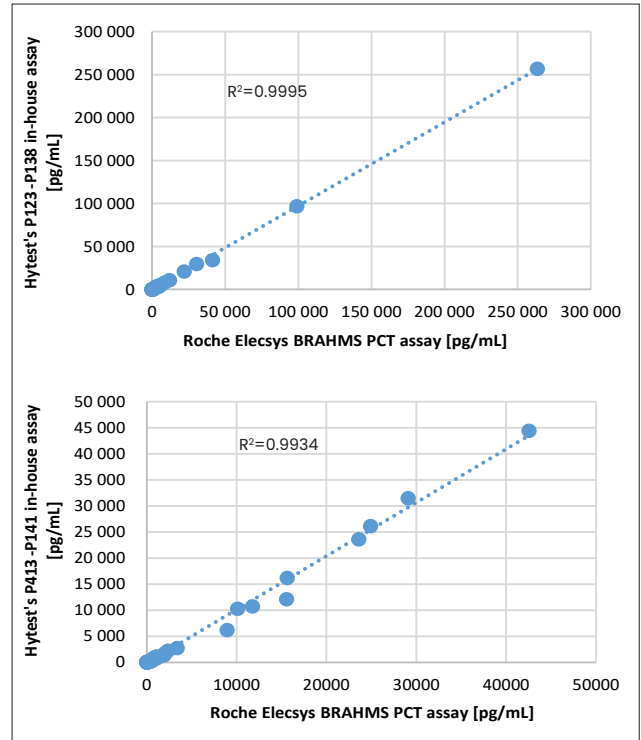


Figure 3.
 Correlation of the developed assays with the Roche Elecsys® BRAHMS PCT assay.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4C10cc* 4C10*	Monoclonal mouse anti-human calcitonin	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+} .

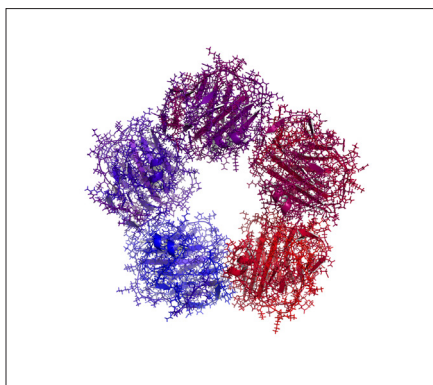


Figure 4.
CRP is a pentamer that is composed of five identical subunits that form a ring like structure.

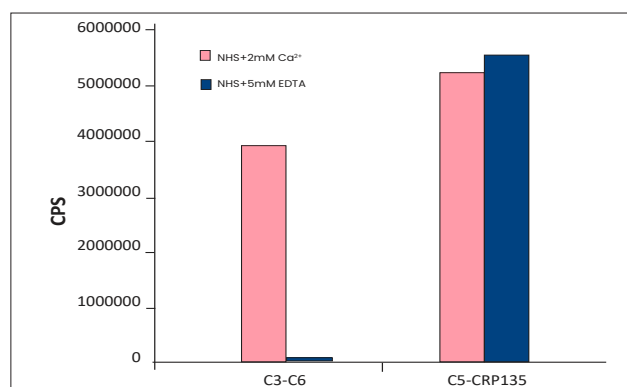


Figure 5.
Influence of EDTA on CRP measurements. Two different MAB pairs were used in a sandwich immunoassay. Pair C3-C6 (left) shows a dependence on Ca^{2+} as it fails to recognize CRP in the presence of EDTA. In contrast, pair C5-CRP135 (right) is unaffected by EDTA in the solution. Normal human serum 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, which therefore enable them to be used in different types of immunoassays.

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.

Recombinant human CRP antigen

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.

De novo hepatic synthesis of CRP starts rapidly after a single systemic stimulus which cause the serum concentrations to rise in approximately 6 hours. Hytest's recombinant human CRP is expressed in mammalian cells and purified in native conditions that excludes renaturation steps and confirms the functional activity of the recombinant protein. The Hytest's Recombinant human CRP is immunochemically active in different sandwich immunoassay pairs using Hytest antibodies, Fig. 6.

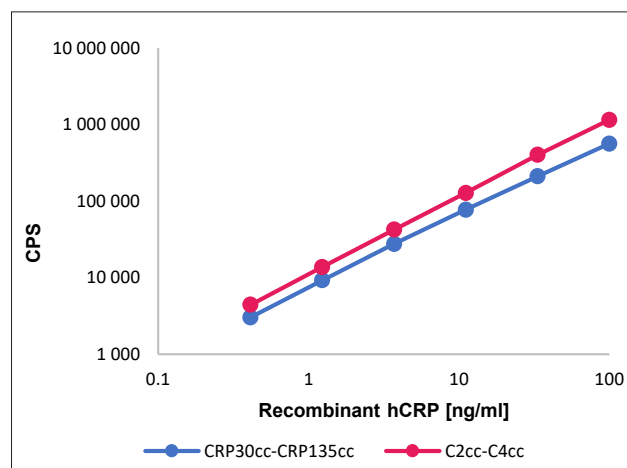


Figure 6. Calibration curve for the recombinant human CRP with CR30cc-CRP135cc and C2cc-C4cc (capture-detection) sandwich immunoassays.

MONOCLONAL ANTIBODIES

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

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

ANTIGEN

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

DEPLETED SERUM

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

Serum Amyloid A (SAA)

CLINICAL UTILITY

- Inflammation
- Tissue injury

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, the biological function of SAA in inflammation is unclear. 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. Therefore, SAA is a non-specific marker of inflammation, and the acute-phase response usually lasts for several days and the concentration of SAA then gradually decreases in the absence of a new stimulus.

SAA can be used in diagnosis, predicting outcomes, and assessing the efficacy of treatment in patients with inflammation. We provide several anti-SAA monoclonal antibodies that are suitable for the development of a quantitative SAA immunoassay for both human and animal diagnostics (Table 1). All our recommended pairs recognize recombinant SAA1, SAA2, and SAA from human blood.

Hytest also provides recombinant human SAA1 (variant SAA1.1, Cat.# 8SA1) and SAA2 (variant SAA2.1, Cat.# 8SA2) expressed in *E. coli*. Both proteins contain an additional N-terminal methionine residue and demonstrate similar immunochemical activity (Figure 7A). In assays using recommended antibody pairs, the dilution curves of recombinant human SAA proteins and human EDTA plasma samples were parallel (Figure 7B). Therefore, SAA concentration can be accurately determined in diluted plasma samples using purified recombinant SAA as a calibrator.

We have tested the cross-reactivity of our recommended antibody pairs with the most common human SAA allelic variants including SAA1.1, SAA1.3, SAA1.5, SAA2.1, and SAA2.2. The prototype immunoassays using our in-house antibody pairs detects all of the mentioned human SAA variants but do not recognize a constitutive isoform SAA4.

Table 1.
Cross-reactivity of Hytest's anti-SAA MAbs.

Cat.#	MAb	Specificity			
		Human SAA	Canine SAA	Equine SAA	Feline SAA
4SA11	A491	+	+	+	+
	A496	+	-	-	-
	VSA6	+	+	+	-
	VSA25	+	+	+	+
	SAA1cc	+	+	-	-
	SAA6	+	+	-	-
	SAA15cc	+	+	-	-
4VS4	SAA19cc	+	+	+	+
	SAA21cc	+	+	+	+
	VSA38cc	+	+	+	+
	VSA31cc	+	+	+	+

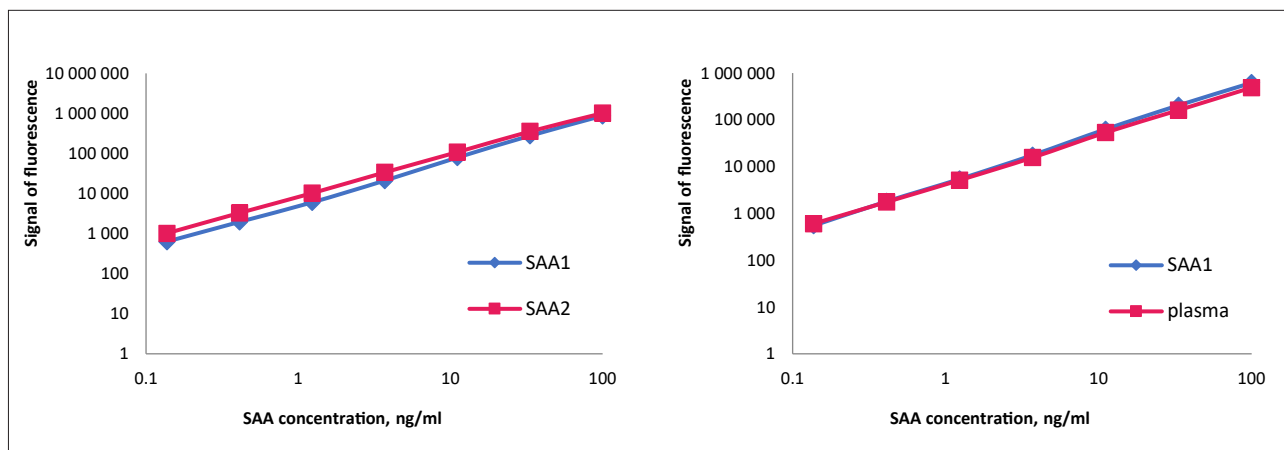


Figure 7. Calibration curves for the human SAA prototype assay using the antibody pairs A496-SAA19cc. A) human recombinant SAA1 and SAA2 were used as the antigens. B) human recombinant SAA1 and EDTA plasma samples were used as the antigens.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4SA11*	Monoclonal mouse anti-serum amyloid A	Enzyme immunoassays Western blotting
4VS4*	Monoclonal anti-serum amyloid A, animal	Enzyme immunoassays

*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%

Interleukin-6 (IL-6)

CLINICAL UTILITY

- Systemic inflammation
- Sepsis

Interleukin-6 (IL-6) participates in inflammation, immune response, and acts in the coordination of developmental, neuronal, and metabolic processes. IL-6 acts as a transmitter of alarm signals to the whole organism, indicating the occurrence of an emergency such as infection or tissue damage. IL-6 acts at the very beginning of the inflammation process, stimulating upregulation of acute-phase proteins such as C-reactive protein, serum amyloid A, fibrinogen, and haptoglobin in hepatocytes. IL-6 also plays an important role in acquired immune response by the stimulation of antibody production and effector T-cell development. The balance between IL-6 interaction with soluble and membrane-bound forms of IL-6 receptor largely determines pro-inflammatory and anti-inflammatory activities of this cytokine.

IL-6 has been shown to be involved in many physiological activities, disease initiation and progression, and the pleiotropic nature of this makes it a key player in many physiologic processes. The determination of IL-6 levels in human blood is primarily accomplished with the use of sandwich type immunoassays. Baseline levels of human IL-6 in the blood are known to be in single pg per ml digits and can increase up to thousands of pg/ml upon severe sepsis. Therefore, assays characterized by high sensitivity and a wide diagnostic window are needed (Figure 8) for the reliable determination of IL-6 in the bloodstream. Hytest provides several monoclonal antibodies that are capable of detecting both recombinant human IL-6 and native IL-6 in serum. All of the developed MAbs are capable of working in a sandwich immunoassay.

IL-6 levels can increase up to 10 ng/ml during severe septic conditions. Therefore, it is important for a clinician to have an opportunity to detect IL-6 concentrations that are high in the immunoassays without prior dilution. The MAb pairs provided by Hytest can offer a wide linearity range together with high sensitivity (see Figure 9).

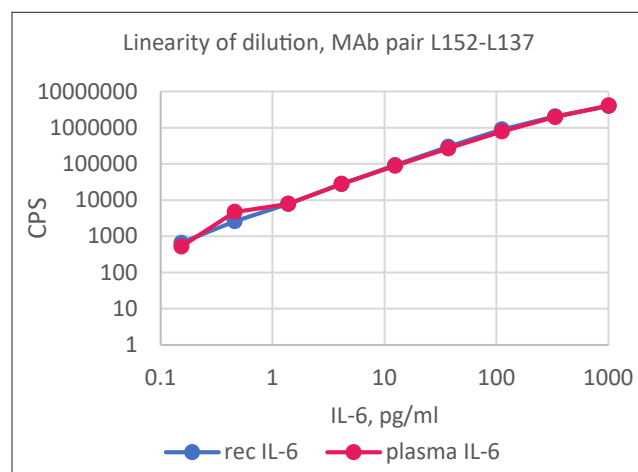


Figure 8. Dilution linearity of recombinant IL-6 and IL-6 from the plasma of septic patients, measured in sandwich CLIA with MAb pair L152-L137. CLIA with Streptavidin-polyHRP was used. Plasma dilution was made in parallel for rec IL-6 and plasma IL-6. Plasma IL-6 concentration was measured with the Roche Cobas 6000 analyzer.

Moreover, the Hytest MAb pairs demonstrates a good correlation in CLIA with the Siemens IMMULITE 2000 IL-6 assay when used for the determination of IL-6 levels in a group of patients (N=107). The testing of another set of clinical samples (N=67) in CLIA with Hytest's MAb pairs using acridinium ester as a label demonstrates an even better correlation with the Roche IL-6 assay (Figure 10).

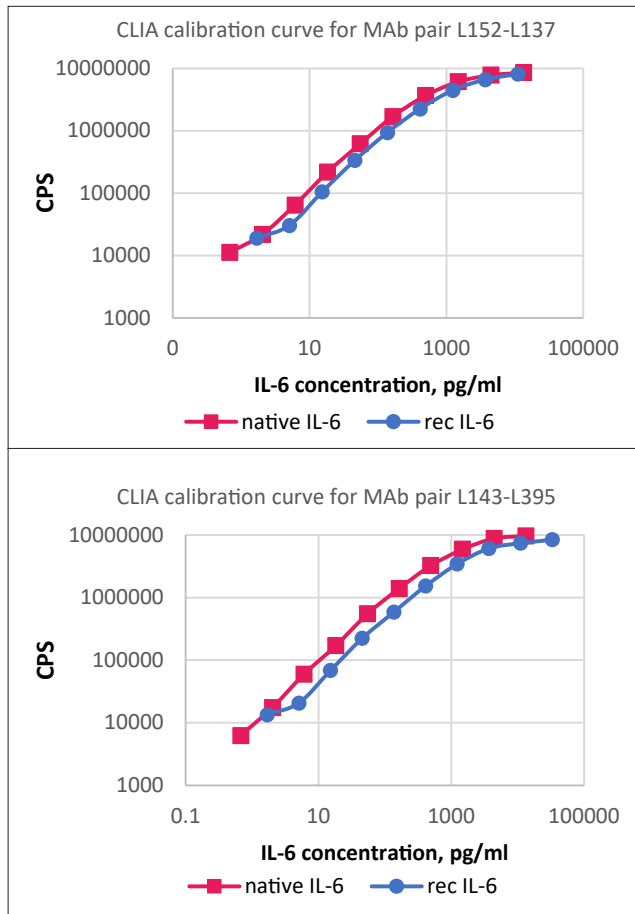


Figure 9. Calibration curve for MAb pairs L152-L137 and L143-L395. CLIA with Streptavidin-polyHRP was used. Recombinant IL-6 and native IL-6 were taken as a calibrator. For native IL-6, mononuclear cells were isolated from the blood of healthy human donors, cultivated in culture, and stimulated with bacterial lipopolysaccharide. The concentration of native IL-6 in conditioned media was determined by the Roche Cobas 6000 analyzer. Coating MAbs 200 ng/well, biotinylated MAbs 100 ng/well. The incubation time was 60 minutes (diluent buffer: PBS+7.5% BSA).

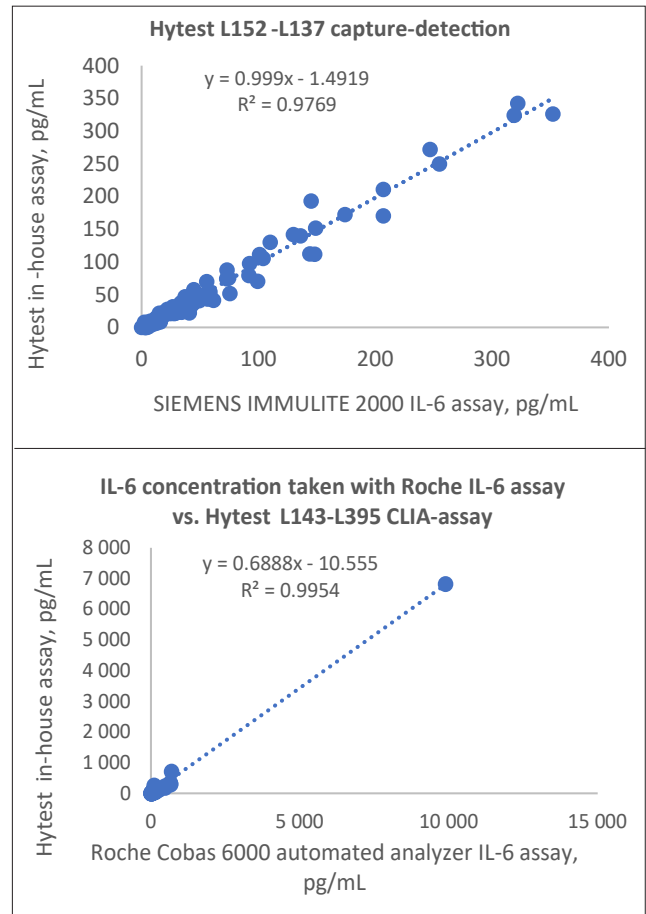


Figure 10. Determination of IL-6 in the clinical samples of patients with the Hytest MAb pairs and correlation to the Siemens IMMULITE 2000 IL-6 and Roche Cobas e602 IL-6 assay. CLIA with acridinium ester as a label was used for the Hytest MAb pairs.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4IL6*	Monoclonal anti-human interleukin 6 (IL-6)	Enzyme immunoassays Lateral flow

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

ANTIGEN

Cat.#	Product	Source	Purity
8IL6	Interleukin 6 (IL-6), recombinant	Recombinant	> 90%

Additional products

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
4I22*	Monoclonal mouse anti-human interferon gamma	Enzyme immunoassays Western blotting

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

Other 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.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4IL12	Monoclonal mouse anti-human interleukin-1, beta	Enzyme immunoassays Immunohistochemistry

Erythropoietin

Erythropoietin is a glycoprotein that is produced by fibroblasts in the kidney as a response to the low oxygen level of blood. Erythropoietin 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.

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.

In addition to its role in erythropoiesis, erythropoietin is also

We provide several monoclonal antibodies specific to erythropoietin.

MONOCLONAL ANTIBODIES

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

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

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HYTEST LTD

Intelligate 1, 6th floor, Joukahaisenkatu 6, FI-20520 Turku, FINLAND

Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**

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