MedixMAB™
INFLAMMATION

Technical Note

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Contents

Introduction

Inflammation is part of the immune system's natural response to infection or tissue injury. Acute inflammation serves as a mechanism of initiating the elimination of infectious agents or damaged tissue by recruiting blood cells to kill invading micro-organisms, which in turn promotes wound healing. Classical symptoms of acute inflammation are redness of the skin, swelling, heat, and pain. If the inflammation persists and becomes chronic, it can eventually cause chronic diseases including atherosclerosis, cancer, Alzheimer's or autoimmune diseases. For this reason, inflammation has started to become one of the most studied areas of medical research.

One of the most common biomarkers for diagnosing inflammation is C-reactive protein (CRP), which is measured from blood samples and indicates whether the inflammation is caused by bacteria. If CRP values rise, doctors will prescribe antibiotics to treat a bacterial infection. If the inflammation is caused by viral infection, CRP levels do not normally increase and therefore antibiotics are not needed. High-sensitivity CRP (hs-CRP) is sometimes used to measure chronic inflammation as it is more precise than standard CRP when measuring baseline (i.e., normal) concentrations of CRP. Recently, other markers such as procalcitonin (PCT) and serum amyloid A (SAA) have also become more widely used for diagnosis of acute inflammation.

Medix Biochemica has concentrated on inflammation biomarkers as a key focus area. For over 35 years, we have continually been at the forefront of monoclonal antibody development, using modern *in vitro* production methods with excellent batch-to-batch consistency.

CRP

C-Reactive protein (CRP) has been named according to its capacity to precipitate the C-polysaccharide of *Streptococcus pneumoniae*. CRP is secreted in response to a variety of inflammatory cytokines, and its main biological role is the activation of the complement system and of other proinflammatory processes. More specifically, CRP binds to phosphocholine and related substances, which are widely distributed in polysaccharides of pathogens and in cellular membranes. CRP therefore recognizes a range of pathogenic targets as well as membranes of damaged and dying host cells, making it one of the key activators of the classical complement pathway.[1-4]

The levels of CRP in blood increase rapidly in response to trauma, inflammation, and infection. It is thus not a specific marker for a particular disease, but rather a widely used indicator of bacterial or fungal infection, or various inflammatory conditions, such as rheumatoid arthritis or inflammatory bowel disease. Normal levels of CRP in blood are generally less than 3 mg/L. In the case of acute inflammation or bacterial infection, CRP levels can rise dramatically, up to 10,000-fold. In recent years, studies have indicated that slightly elevated CRP levels might be linked to several other disease types as well, such as cardiovascular diseases or cancer. However, the physiological connection between CRP levels and the risk of cardiovascular events or malignant tumors is not yet completely understood.[5-6]

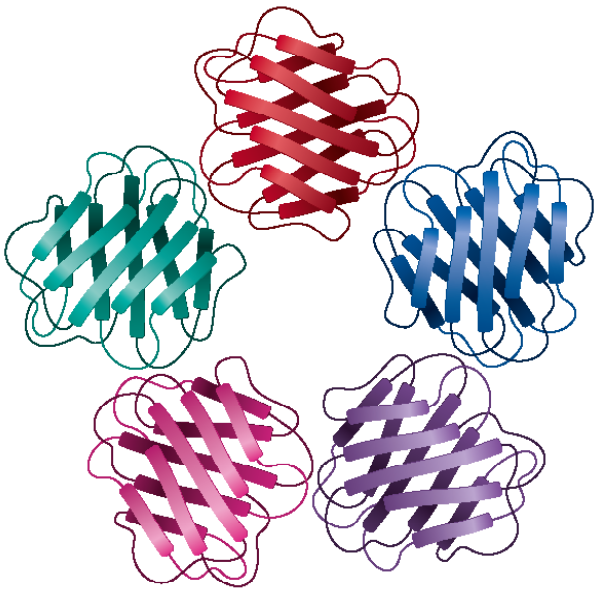
The human CRP molecule is a pentamer, i.e. it is composed of five identical polypeptide subunits, each containing 206 amino acid residues and having a molecular mass of 23 kDa. It belongs to pentraxins, which are an evolutionary conserved family of proteins with a unique architecture – a planar, doughnut-shaped ring.[7]

Medix Biochemica's extensive experience on monoclonal antibodies used in CRP diagnostics extends back to the 1980's when our first products – Anti-human CRP mouse monoclonal antibodies 6404 and 6405 – were developed. Since then, the product family has been extended, and it currently includes five different mouse monoclonal antibodies recognizing CRP.

The CRP antibodies have been studied using multiplexed surface plasmon resonance (SPR) technology and ELISA to identify good antibody pairs for CRP detection. It should be noted that the functionality of two antibodies as a pair is dependent on the method and conditions used in the assay.

The association and dissociation characteristics of an antibody-antigen binding reaction can have significant effects on the usability of antibodies in IVD applications. The association rate constant (on-rate) and the dissociation rate constant (off-rate) for Medix Biochemica's anti-CRP antibodies have been measured using SPR. Anti-human CRP antibodies have no cross-reactivity with the serum amyloid P (SAP) component.

Scientific publications: page 14



The human CRP molecule is a pentamer.

Anti-human CRP monoclonal antibodies

CRP antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
6402	100145	5	24	IgG_1	ELISA, IT, LF
6403	100146	5	36	IgG_1	ELISA, IT, LF
6404	100058	2.0–2.5	24	IgG_1	ELISA, IT, LF, WB
6404	100061	> 6	24	IgG_1	ELISA, IT, LF, WB
6405	100358	5	18	IgG_1	ELISA, IT, LF, WB
6407	100147	5	24	IgG_1	ELISA, IT, LF

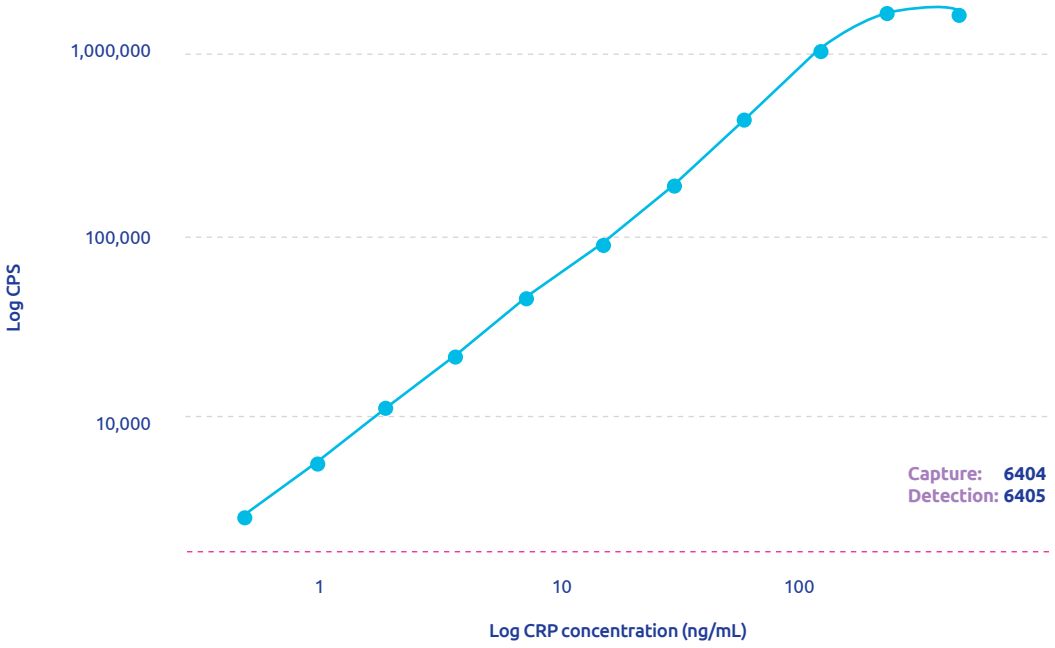
Pair recommendations

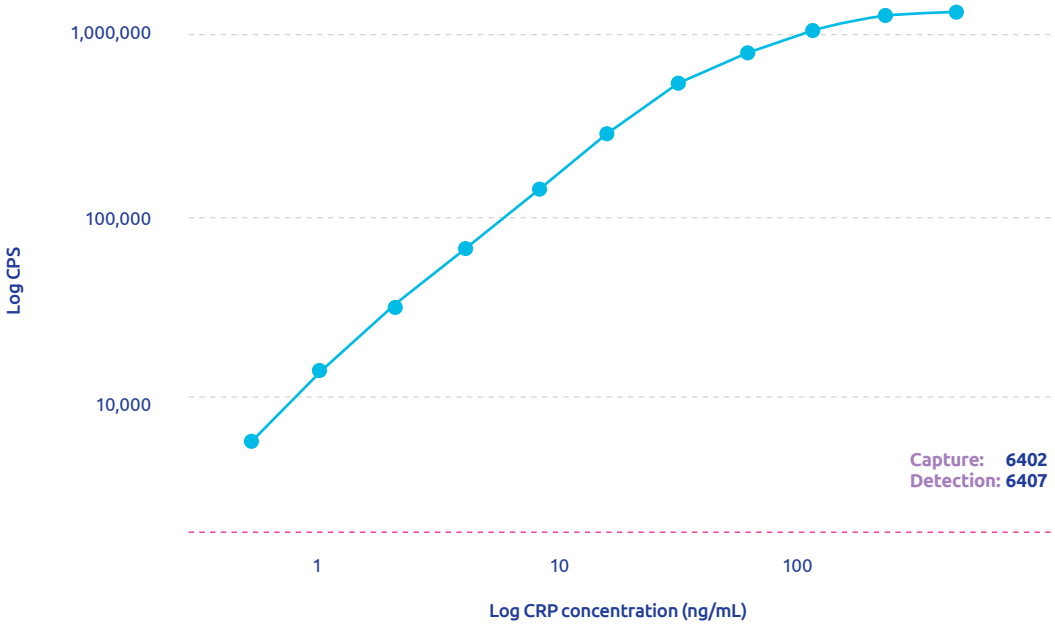
		Detection				
		6402	6403	6404	6405	6407
Capture	6402	+	+	–	+	+
	6403	+	+	–	+	–
	6404	–	–	+	+	–
	6405	+	–	–	+	–
	6407	+	–	–	+	+

Kinetic parameters

CRP antibody	Association rate constant, k_{on} (1/Ms)	Dissociation rate constant, k_{off} (1/s)	Dissociation constant, K_D (M)
6402	7.3×10^5	1.2×10^{-4}	1.6×10^{-10} = 0.16 nM
6403	9.6×10^5	1.6×10^{-4}	1.6×10^{-10} = 0.16 nM
6404	7.7×10^6	4.1×10^{-4}	5.3×10^{-11} = 0.05 nM
6405	2.6×10^6	6.6×10^{-5}	2.6×10^{-11} = 0.03 nM
6407	1.1×10^6	1.3×10^{-4}	1.1×10^{-10} = 0.11 nM

CRP standard curves





SAA

Acute-phase serum amyloid A (SAA) proteins are synthesized in response to inflammatory signals, similar to CRP. SAA1 and SAA2 – the two major types of SAA found in humans – are highly homologous proteins with a molecular mass of about 12 kDa. When released into circulation, SAAs associate with high-density lipoprotein (HDL) particles. Several immunological roles have been suggested for SAAs, such as the induction of cytokine synthesis and recruitment of immune cells to inflammatory sites, however the precise physiological function of SAAs is still not fully understood.[8-10]

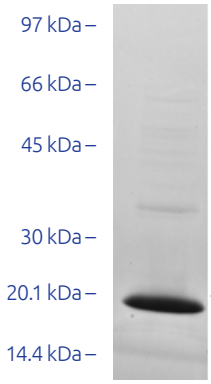
SAA concentrations in blood increase within a few hours after the onset of inflammation. The normal levels vary from <2–8 mg/L, and the concentration can increase by as much as 1000-fold during acute inflammation. Like CRP, SAA can therefore be used as an indicator for microbial infections or for various inflammatory conditions. An increasing amount of evidence has also been collected about the significance of chronic inflammation in the development and progression of many other severe diseases, such as various cancers and cardiovascular diseases, and SAA has been suggested to be a good prognostic marker for these patients.[11-13] In addition, as SAA proteins are highly conserved in vertebrates, SAA measurements are also used in veterinary diagnostics.

Medix Biochemica offers two mouse monoclonal antibodies for SAA detection, which can be used as a pair in diagnostic tests.

In addition to antibodies, Medix Biochemica offers a recombinant human SAA protein antigen, product code 610070, which contains the amino acids 19–122 (according to UniProt #P0DJI8; no signal peptide) and an N-terminal histidine tag.

Purity

Denaturing SDS-PAGE analysis of recombinant human SAA protein antigen.



Anti-human SAA monoclonal antibodies and recombinant antigen

SAA antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
2201	100279	5	18	IgG_{2a}	ELISA, LF, IT
2203	100289	5	18	IgG_1	ELISA, LF, IT

SAA antigen	Product code
Recombinant SAA, 100 µg	610070

Kinetic parameters

SAA antibody	Association rate constant, k_{on} (1/Ms)	Dissociation rate constant, k_{off} (1/s)	Dissociation constant, K_D (M)
2201	1.9×10^5	1.8×10^{-4}	9.6×10^{-10} = 1.0 nM
2203	1.1×10^5	3.0×10^{-4}	2.8×10^{-9} = 2.8 nM

Calprotectin

Calprotectin is a calcium-binding heterodimer formed by two proteins, S100A8 and S100A9, associating together. It has a molecular mass of 24 kDa, and each subunit binds two calcium ions. In the presence of abundant calcium, two calprotectins may also bind together to form heterotetramers.[19]

Calprotectin is secreted by neutrophils at the site of inflammation. The protein has been shown to play a role in regulating cytokine synthesis, and also possesses antimicrobial activity mediated through the sequestration of essential nutrient metals such as zinc and manganese.[20-21]

Calprotectin levels in plasma and stool are significantly increased in infectious and inflammatory conditions. Analysis of fecal calprotectin is commonly used to diagnose intestinal inflammations, especially inflammatory bowel disease (IBD). Information about fecal calprotectin levels provides valuable support for treatment decisions as it can consistently differentiate IBD from irritable bowel syndrome.[22]

Medix Biochemica offers five different monoclonal antibodies for sensitive calprotectin detection, with specificities for both S100A8/9 subunits. In addition, Medix Biochemica's product portfolio includes three types of recombinant calprotectin antigens.

Anti-human calprotectin monoclonal antibodies and recombinant antigens

Calprotectin antibody	Product code	Calprotectin subunit binding specificity	Shelf life (months at +2–8°C)	Subclass	Applications tested
3403	100460	S100A9	12	IgG_{2a}	ELISA, LF
3404	100468	S100A8	24	IgG_1	ELISA, LF
3405	100469	S100A8	N/D	IgG_1	ELISA, LF
3406	100470	S100A8	24	IgG_1	ELISA, LF
3407	100618	S100A8/S100A9 complex	18	IgG_1	ELISA, LF

Calprotectin antigens	Product code	Subunit structure
Recombinant Calprotectin, 100 µg	610061	Heterodimer S100A8/9
Recombinant Calprotectin S100A8, 50 µg*	710018	S100A8 subunit
Recombinant Calprotectin S100A9, 50 µg*	710019	S100A9 subunit

* Also available in 500 µg and 1000 µg package sizes.

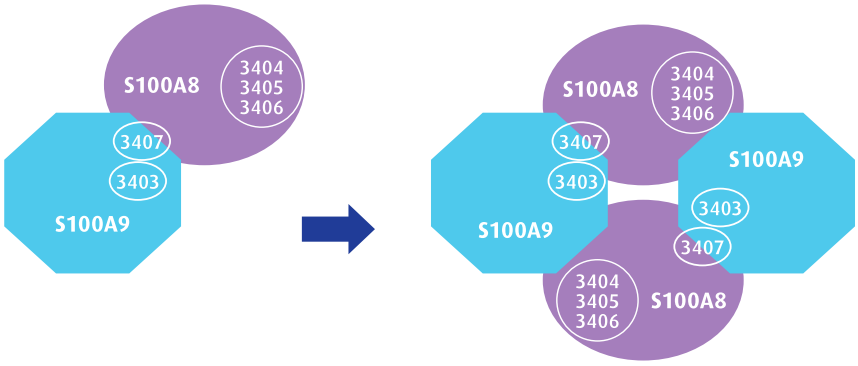
Pair recommendations

Capture \ Detection	3403	3404	3405	3406	3407
3403	–	+	+	+	–
3404	+	–	–	–	+
3405	+	–	–	–	+
3406	+	–	–	–	+
3407	–	+	+	+	+

Kinetic parameters

Calprotectin antibody	Association rate constant, k_{on} (1/Ms)	Dissociation rate constant, k_{off} (1/s)	Dissociation constant, K_D (M)
3403	1.9×10^{6}	9.1×10^{-4}	$4.8 \times 10^{-10} = 0.5$ nM
3404	4.8×10^{5}	6.1×10^{-5}	$1.3 \times 10^{-10} = 0.1$ nM
3405	7.4×10^{5}	1.5×10^{-4}	$2.0 \times 10^{-10} = 0.2$ nM
3406	7.6×10^{5}	1.1×10^{-4}	$1.4 \times 10^{-10} = 0.1$ nM
3407	4.0×10^{4}	5.0×10^{-5}	$1.3 \times 10^{-9} = 1.3$ nM

Binding properties



- 3403 recognizes both the S100A9 subunit and the S100A8/S100A9 complex
- 3404, 3405 and 3406 recognize the S100A8 subunit and the S100A8/S100A9 complex
- 3407 only recognizes the S100A8/S100A9 complex but not the individual subunits.

PCT

Procalcitonin (PCT) is a precursor protein of the hormone calcitonin, produced by the thyroid gland. It is a 116 amino acid protein that is cleaved intracellularly to form three peptides: N-terminal procalcitonin, calcitonin, and katacalcin. In healthy individuals, only the calcitonin polypeptide is secreted into the bloodstream, and PCT serum levels are very low. However, in patients with systemic infections, and especially sepsis, PCT is secreted by several cell types, not only in the thyroid gland, and levels rise rapidly. High PCT levels have also been identified in patients suffering from cardiogenic shock, severe systemic inflammatory response syndrome (SIRS), and trauma.[14-16]

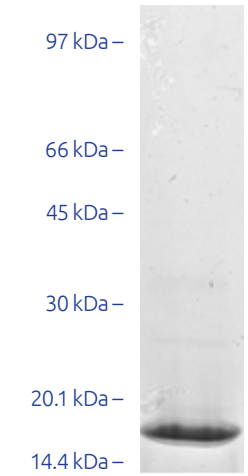
The immunological roles of PCT have been shown to be linked to the neutralization of bacterial lipopolysaccharides (LPS) and to the reduction of cytokine release. The LPS are strong endotoxins and mediators of septic shock, and it has been suggested that PCT may neutralize LPS via the polycationic motifs present in its molecular structure.[17]

The association of elevated serum PCT levels with bacterial infections makes PCT a very promising biomarker for sepsis, as it can be used to discriminate bacterial from non-infective causes of systemic inflammations. Additionally, it has been suggested that PCT measurement is useful in the management of antibiotic therapy, which is becoming increasingly important due to the spread of antibiotic-resistant pathogens.[15,16,18]

Medix Biochemica has several monoclonal antibodies for PCT, against both katacalcin and calcitonin subunits. We also offer the full-length PCT antigen.

Purity

Denaturing SDS-PAGE analysis of recombinant human PCT protein antigen.



Anti-human PCT monoclonal antibodies and recombinant antigen

PCT antibodies	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Specificity	Applications tested
4003	100562	5	N/D	IgG_1	Katacalcin	ELISA, LF
4004	100563	5	24	IgG_1	Calcitonin	ELISA, LF
4005	100564	5	24	IgG_1	Calcitonin	ELISA, LF
4006	100567	5	N/D	IgG_1	Katacalcin	ELISA, LF
4008	100769	5	N/D	IgG_3	Katacalcin	ELISA, LF
4051	700020	>1	60	IgG_1	Calcitonin	ELISA, LF

PCT antigen	Product code
Recombinant PCT, 100 µg	610080

Pair recommendations

Capture \ Detection	4003	4004	4005	4006	4008	4051
4003	–	+	+	–	–	+
4004	+	–	–	+	+	–
4005	+	–	–	+	+	–
4006	–	+	+	–	–	+
4008	–	+	+	–	–	+
4051	+	–	–	+	+	–

Kinetic parameters

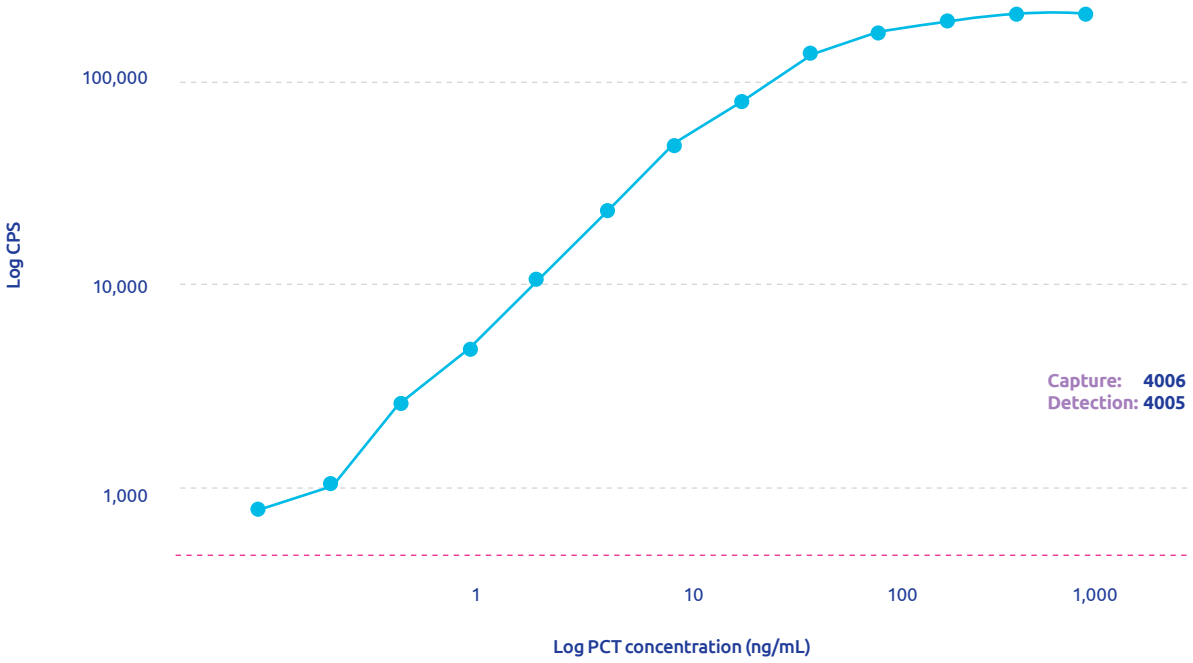
PCT antibody	Association rate constant, k_{on} (1/Ms)	Dissociation rate constant, k_{off} (1/s)	Dissociation constant, K_D (M)
4003	4.8×10^{4}	2.2×10^{-4}	4.6×10^{-9}
4004*	2.5×10^{4}	-	-
4005*	2.9×10^{4}	-	-
4006	6.7×10^{4}	4.0×10^{-4}	5.2×10^{-10}
4008	N/D	N/D	N/D
4051	N/D	N/D	N/D

*The antibody does not dissociate in the reaction conditions used.

PCT binding properties

N-terminal part	Calcitonin	Katacalcin	
	4004	4003	4006
	4005		4008
	4051		

PCT standard curve



NGAL

Neutrophil gelatinase-associated lipocalin (NGAL) is a small glycoprotein belonging to the superfamily of lipocalins. NGAL is expressed in epithelial tissues associated with anti-microbial defense, and in the distal tubules and collecting duct of the kidney. Elevated levels of blood and urine NGAL are associated with acute or chronic renal failure, and elevated serum levels with active inflammatory bowel disease (IBD) and cardiovascular events.

Medix Biochemica offers four mouse monoclonal antibodies for NGAL detection as well as a recombinant NGAL antigen. The protein is produced in *Escherichia coli* host cells and contains the amino acids 21–198 (according to UniProt #P80188) and a C-terminal histidine tag.

Anti-human NGAL monoclonal antibodies and recombinant antigen

NGAL antibody	Product code	Concentration (mg/mL)	Shelf life (months at +2–8°C)	Subclass	Applications tested
4202	100579	5	24	IgG_1	ELISA, IT
4203	100580	5	24	IgG_1	ELISA, IT
4204	100581	5	36	IgG_1	ELISA, IT
4205	100582	5	24	IgG_1	ELISA, IT

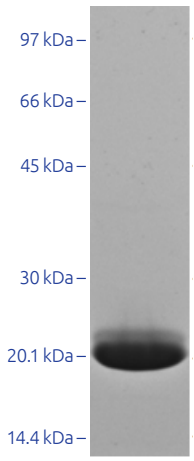
NGAL antigen	Product code
Recombinant NGAL, 100 µg	610012

Pair recommendations

Capture \ Detection	4202	4203	4204	4205
4202	–	+	+	+
4203	+	–	+	+
4204	+	+	–	+
4205	+	+	+	–

Purity

Denaturing SDS-PAGE analysis of recombinant human NGAL protein antigen.

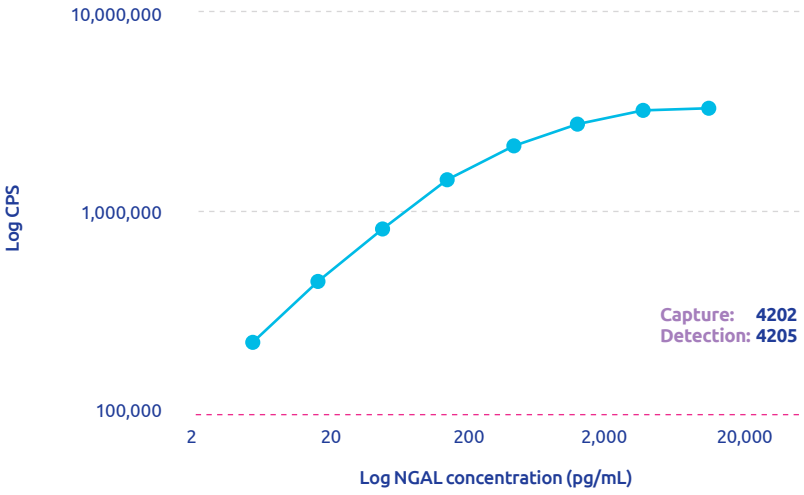


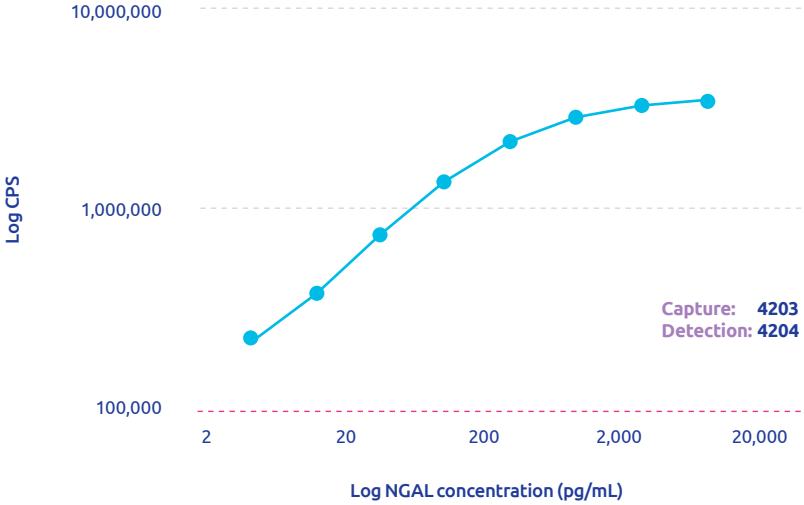
Kinetic parameters

NGAL antibody	Association rate constant, k_{on} (1/Ms)	Dissociation rate constant, k_{off} (1/s)	Dissociation constant, K_D (M)
4202	6.4×10^{5}	4.0×10^{-4}	6.3×10^{-10}
4203*	2.2×10^{5}	-	-
4204	1.8×10^{5}	3.4×10^{-5}	1.8×10^{-10}
4205	5.6×10^{4}	4.9×10^{-5}	8.6×10^{-10}

*The antibody does not dissociate in the reaction conditions used.

NGAL standard curves





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Scientific articles describing the use of MedixMAB CRP antibodies include:

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- Elschner T, Scholz F, Miethe P and Heinze T (2014) Rapid Flow Through Immunoassay for CRP Determination Based on Polyethylene Filters Modified with ω–Aminocellulose Carbamate. Macromol Biosci. 14:1539-1546.
- Tarkkinen P, Palenius T and Lövgren T (2002) Ultrarapid, ultrasensitive one-step kinetic immunoassay for C-reactive protein (CRP) in whole blood samples: Measurement of the entire CRP concentration range with a single sample dilution. Clin Chem. 48:269-277.

References:

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4. Venugopal SK, Devaraj S & Jialal I (2005) Macrophage Conditioned Medium Induces the Expression of C-Reactive Protein in Human Aortic Endothelial Cells : Potential for Paracrine/Autocrine Effects. Am J Pathol. 166:1265–1271.
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More information on our products
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CPS = Counts per second
CLIA = Chemiluminescence immunoassay
ELISA = Enzyme-linked immunosorbent assay
IT = Immunoturbidimetry
LF = Lateral flow
N/A = Not Applicable
N/D = Not Determined
WB = Western blot