

# Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a protein cytokine that was discovered in the 1980s. It is also known as B-cell stimulatory factor 2, hepatocytestimulating factor, hybridoma growth factor, or interferon (IFN)- $\beta$ 2. IL-6 participates in inflammation, immune response, and acts in the coordination of developmental, neuronal, and metabolic processes (1). 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. Human IL-6 is made up of 212 amino acids, including a 28-amino-acid signal peptide, and its gene has been mapped to chromosome 7p21. Although the core protein is 20 kDa, glycosylation accounts for the size of 21–26 kDa of natural IL-6.

Interestingly, IL-6 can exert pro-inflammatory as well as anti-inflammatory signals, depending on the receptor complex with which it interacts. IL-6 interacts with IL-6 receptor  $\alpha$  and this binary complex then further binds to gp 130. The resulting hexameric complex is capable of downstream signaling. The IL-6 receptor  $\alpha$  can function as membrane-bound proteins and also exist in soluble form (2). Depending on the form of IL-6 receptor, IL-6 can transmit anti-inflammatory messages (by binding of IL-6 to IL-6 receptor  $\alpha$  in the cell membrane) or pro-inflammatory ones (by binding to a soluble form of IL-6 receptor  $\alpha$ ). Gp130 is a membrane-bound co-receptor that is expressed in various cell types whereas IL-6 receptor exists in membrane-bound form only on certain cell types, such as hepatocytes, neutrophils, T-cells, or monocytes (2).

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 (3).

## Clinical value of IL-6

IL-6 has been shown to be involved in many physiological activities, disease initiation and progression, and the pleiotropic nature of this cytokine makes it a key player in many physiologic processes (4). IL-6 is involved in hematopoiesis, and neuronal cells proliferation (5-6). Atherosclerosis is believed to include inflammation processes so that IL-6 has been used as a marker of cardiovascular risk (7). Increased IL-6 levels are strongly correlated with hypertension, dyslipidemia, and glucose resistance (8).

It came as no surprise that serum IL-6 levels were also upregulated during the recent outbreak of the latest Coronavirus infection (SARS-CoV-2). According to Gong et al., IL-6 levels were significantly lower in mild cases compared with severe cases and critically ill groups of patients with SARS-CoV-2 (9). IL-6 levels are associated with the severity of the COVID-19 infection (10-11). Moreover, IL-6 could be a predictive marker of survival in COVID-19 patients outperforming CRP, D-dimer, and ferritin, independently of demographics and comorbidities (12). 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 (13). Therefore, assays characterized by high sensitivity and a wide diagnostic window are needed for the reliable determination of IL-6 in the bloodstream.

## CLINICAL UTILITY

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

## Reagents for the IL-6 immunoassay development

Hyttest provides several monoclonal antibodies that are capable of detecting both recombinant human IL-6 (Cat.# 8IL6) and native IL-6 in serum.

Monoclonal antibodies were developed using full-length human recombinant IL-6 as an immunogen and mice, rats, and rabbits as the source of the immune cells. All of the developed MABs are capable of working in a sandwich chemiluminescent assay with streptavidin-polyHRP. The recommended MAB pairs are described in Table 1, 2 and 3.

**Table 1. Recommended MAB pairs to be used in a chemiluminescent sandwich ELISA with Streptavidin-polyHRP (15 minute assay time).**

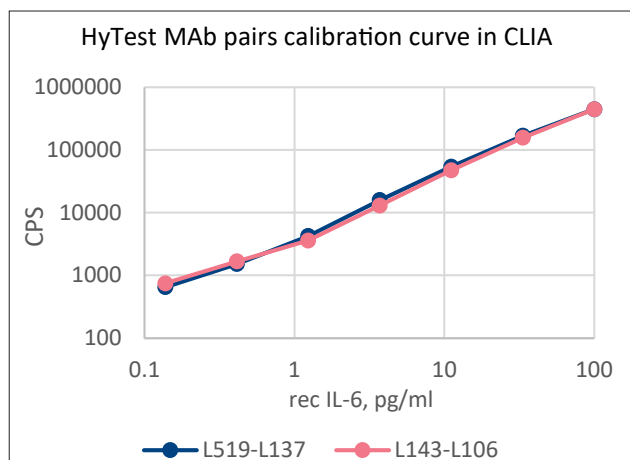
Coating antibody	Detector antibody	LoD, pg/ml
L152	L137	0.7
L143	L395	0.4
L519	L395	0.7
L143	L106	0.4
L152	L395	0.5

**Table 2. Recommended MAB pairs to be used in a chemiluminescent sandwich ELISA with Acridinium ester or Alkaline Phosphatase.**

Coating antibody	Detector antibody
L152	L137
L152	L106
L143	L106

**Table 3. Recommended MAB pairs to be used in a lateral flow assay with fluorescent label.**

Coating antibody	Detector antibody
L395	L152
L143	L395



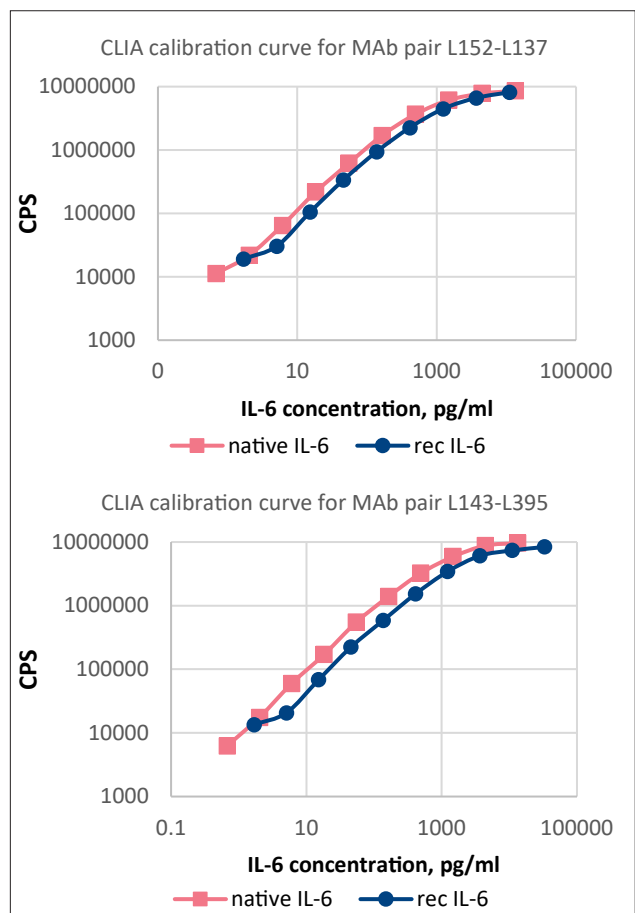
**Figure 1.** Calibration curve of Hyttest MAB pairs L519-L137 and L143-L106 (capture-detection) with recombinant IL-6. CLIA with Streptavidin-polyHRP was used. Coating MABs 200 ng/well, biotinylated MABs 50 ng/well for L137 and 100 ng/well for L106. The incubation time was 15 minutes (diluent buffer: PBS+7.5% BSA).

Hyttest's MAB pairs demonstrate excellent sensitivity when used for the determination of recIL-6 concentration in 1-100 pg/ml range (see Figure 1).

IL-6 levels can increase up to 10 ng/ml during severe septic conditions (14). 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 Hyttest can offer a wide linearity range together with high sensitivity (see Figure 2).

When recombinant IL-6 is titrated in a sandwich CLIA along with native IL-6, both titration curves go in parallel. This indicates that the interaction of MAB pairs with recombinant IL-6 is similar to that of native IL-6 (see Figure 3).

To test Hyttest MAB pairs in a clinical setting, we used a collection of blood samples of septic patients, both serum and plasma (N=40), and we determined IL-6 levels using several Hyttest MAB pairs (see Figure 4). Correlation analysis of data collected with IL-6 levels was determined in the same set of samples with the Roche Cobas 6000 IL-6 assay (correlation coefficient  $R^2=0.9954$ ). Hyttest's MAB pairs could be used for detecting IL-6 levels both in human serum and human plasma, and they demonstrate quantitative results that correlate well with IL-6 data collected using the Roche Cobas 6000 analyzer.

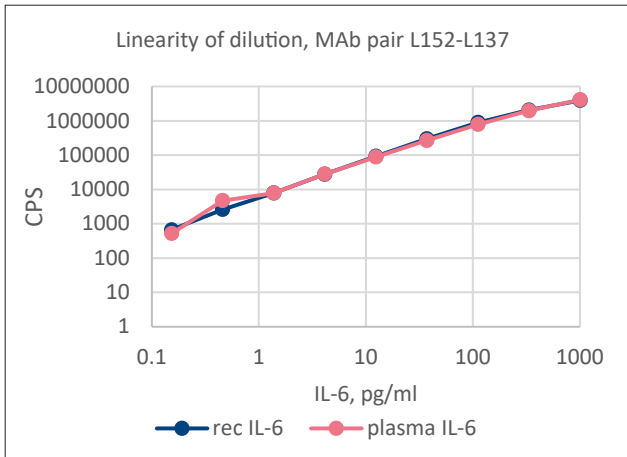


**Figure 2.** Calibration curve for MAB pairs L152-L137 and L143-L395. CLIA with Streptavidin-polyHRP was used. Recombinant IL-6 and native IL-6 were taken as a calibrator. For native IL-6, mononuclear cells were isolated from the blood of healthy human donors, cultivated in culture, and stimulated with 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).

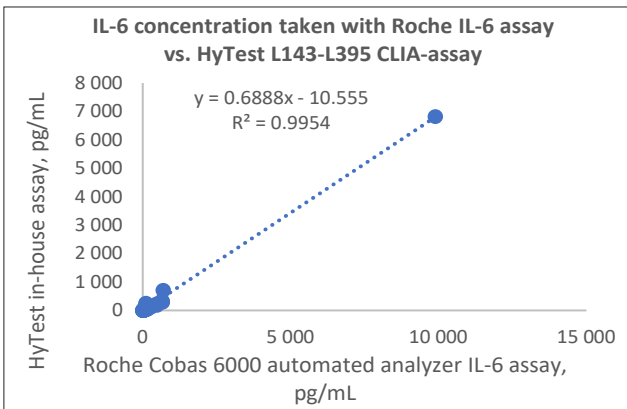
The testing of another set of clinical samples (N=67) in CLIA with Hytest's MAb pairs using acridinium ester as a label demonstrated an even better correlation with the Roche IL-6 assay (see Figure 5).

Moreover, the Hytest MAb pair L152-L137 demonstrated 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) (see Figure 6).

To check for cross-reactivity, a panel of cognate human proteins was used. IL1 $\alpha$ , IL1 $\beta$ , IL2, IL3, IL4, IL8, INF $\gamma$ , TNF $\alpha$  at a concentration of 50 ng/ml were used for cross-reactivity testing in sandwich CLIA. For all of the MAb pairs tested, the cross-reactivity level did not exceed 0.11%.

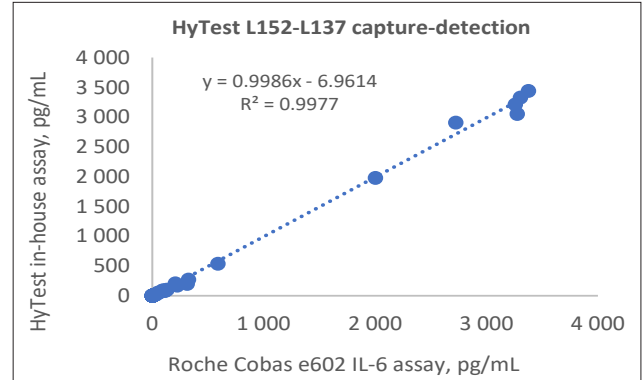


**Figure 3.** Dilution linearity of recombinant IL-6 and IL-6 from the plasma of septic patients, measured in sandwich CLIA with MAb pair L152-L137. CLIA with Streptavidin-polyHRP was used. Plasma dilution was made in parallel for rec IL-6 and plasma IL-6. Plasma IL-6 concentration was measured with the Roche Cobas 6000 analyzer.

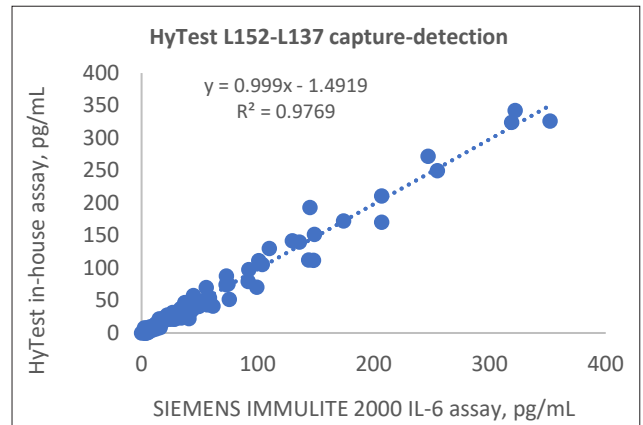


**Figure 4.** Testing of the blood samples of septic patients with Hytest's MAb pair L143-L395 and the comparison to IL-6 mass concentration was determined in the same set of samples with the Roche Cobas 6000 IL-6 assay. CLIA with Streptavidin-polyHRP was used. Undiluted (all of the samples but one) serum or plasma were used in assays. Recombinant IL-6 was used as a calibrator in Hytest's L143-L395 assay. Coating MAbs 200 ng/well, biotinylated MAbs 100 ng/well (diluent buffer: PBS+7.5% BSA). The incubation time was 1 hour for the Hytest MAb pair. The Roche Cobas 6000 IL-6 assay was used according to the manufacturer's instructions.

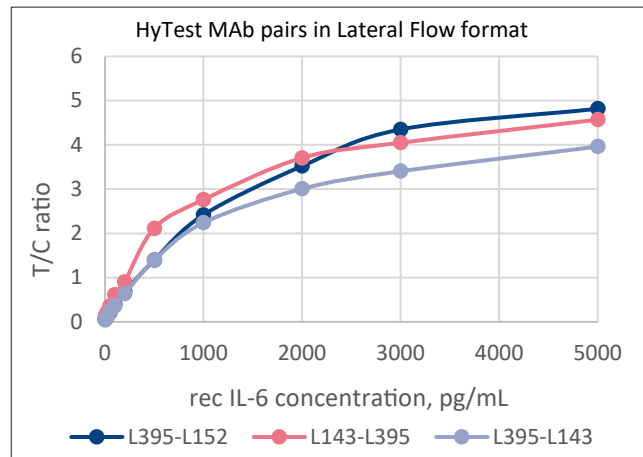
Hytest's MABs could be used to construct Lateral Flow assays for the quantitative determination of IL-6 (see Figure 7).



**Figure 5.** Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L152-L137 and correlation to the Roche IL-6 data. CLIA with acridinium ester as a label was used for the Hytest MAb pair.

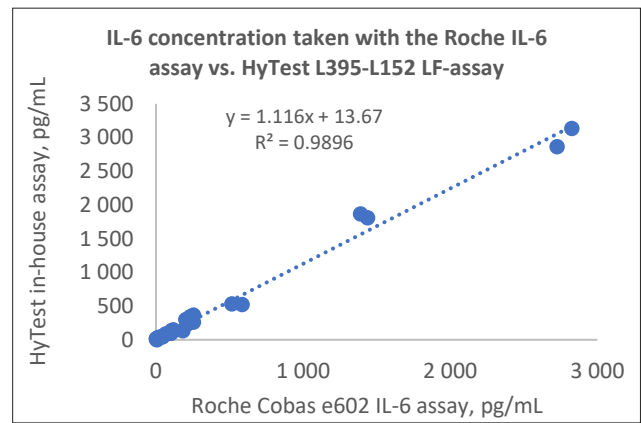


**Figure 6.** Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L152-L137 and correlation to the Siemens IMMULITE 2000 IL-6 data. CLIA with acridinium ester as a label was used for the Hytest MAb pair.



**Figure 7.** Calibration curve for selected Hytest MAB pairs in a Lateral Flow assay.

The concentration of serum IL-6 in the clinical samples of patients determined with the Hytest MAb pairs correlate well with the Roche assay, when taken in Lateral Flow format (see Figure 8).



**Figure 8.**  
Determination of IL-6 in the clinical samples of patients with the Hytest MAb pair L395-L152 and correlation to the Roche IL-6 data. Lateral Flow (LF) was used for the Hytest MAb pair.

## REFERENCES

- Tanaka et al.** IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol 2014; 6: a016295.
- Reeh, H., Rudolph, N., Billing, U. et al.** Response to IL-6 trans- and IL-6 classic signaling is determined by the ratio of the IL-6 receptor  $\alpha$  to gp130 expression: fusing experimental insights and dynamic modelling. 2019, Cell Commun Signal 17, 46.
- Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK.** Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. 2006, Nature 441: 235–238.
- Del Giudice M and Gangestad SW.** Rethinking IL 6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. 2018, Brain Behav Immun 70: 61–75.
- Audet J, Miller CL, Rose-John S, et al.** Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic stem cells. 2001, Proc Natl Acad Sci U S A. 98(4): 1757–62.
- März P, Otten U, Rose-John S.** Neural activities of IL-6-type cytokines often depend on soluble cytokine receptors. 1999, Eur J Neurosci. 11(9): 2995–3004.
- Swerdlow DI, Holmes MV, Kuchenbaecker KB, Engmann JE, Shah T, Sofat R, Guo Y, Chung C, Peasey A, et al.** The interleukin-6 receptor as a target for prevention of coronary heart disease: A mendelian randomisation analysis. 2012, Lancet 379: 1214–1224,
- Eder K, Baffy N, Falus A and Fulop AK.** The major inflammatory mediator interleukin-6 and obesity. 2009, Inflamm Res 58: 727–736,
- Gong J, Dong H, Xia SQ, et al.** Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19 pneumonia [published online February 27, 2020]. medRxiv. doi:10.1101/2020.02.25.20025643.
- Ulhaq, Z.S., Soraya, G.V.** Interleukin-6 as a potential biomarker of COVID-19 progression. 2020, Med. Mal. Infect. 50, 382–383.
- Mojtabavi H, Saghazadeh A, Rezaei N.** Interleukin-6 and severe COVID-19: a systematic review and meta-analysis. 2020, Eur.Cytokine Netw. 31(2): 44–49.
- Diane Marie Del Valle et al.** An inflammatory cytokine signature predicts COVID-19 severity and survival. 2020, Nature Medicine 26, 1636–1643.
- Song, M., and Kellum, J.A.** Interleukin-6. 2005, Crit Care Med 33, 12, S463–S465.
- Huang, L., Zhao, X., Qi, Y. et al.** Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. 2020. Cell Mol Immunol 17, 1092–1094.

## ORDERING INFORMATION

### MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Interleukin 6 (IL-6)	4IL6	L106	IgG1	<i>In vitro</i> , EIA, LF
		L137	IgG2a	<i>In vitro</i> , EIA, LF
		L143	IgG1	<i>In vitro</i> , EIA, LF
		L152	IgG1	<i>In vitro</i> , EIA, LF
		L395	IgG	EIA, LF, recombinant rabbit antibody
		L519	IgG1	EIA, recombinant chimeric antibody

### ANTIGEN

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