

PeliKine compact™ human IL-6

Product number M1916

Introduction

Interleukin 6 (IL-6) is a mediator of the inflammatory response and is involved in the induction of acute phase proteins [1,2,3,4] and the development of fever [5]. A marked correlation between IL-6 levels and inflammatory processes has been demonstrated in synovial fluid and serum of rheumatoid arthritis patients [6,7,8] and in serum of patients with burns [9,10]. It was demonstrated that in recipients of kidney transplants the IL-6 levels in serum and urine hallmark the onset of rejection episodes [11,12]. Elevated IL-6 levels were also observed in sera of patients with septic shock, multiple myeloma and alcoholic hepatitis, and a significant difference between IL-6 levels of survivors and non-survivors was found [13,14,15].

Bioassays for the quantification of IL-6, based on the proliferation of B-cell hybridomas have been used for several years [16,17,18]. These assays, although sensitive, are time consuming and susceptible to interference by other substances. This PeliKine compact™ IL-6 ELISA kit [19] has been developed for faster, more reproducible and specific quantification of human IL-6 (hIL-6) in plasma and other body fluids, as well as in cell-culture supernatant.

Assay procedure

See Assay procedure for PeliKine compact™ ELISA kit: www.sanquinreagents.com → Products → Cytokines → Compact cytokine kits → on bottom of page → 'optimized assay procedure'.

Kit component list

Quantity	Kit component	Volume	Cap colour	
1 vial	coating antibody	100-fold concentrated	375 µl	red
1 vial	blocking reagent	50-fold concentrated	2 ml	transparent
1 vials	IL-6 standard	see label	750 µl	black
1 vial	biotinylated antibody	100-fold concentrated	375 µl	yellow
1 vial	streptavidin-poly-HRP conjugate	10,000-fold concentrated	20 µl	brown
1 bottle	HPE-dilution buffer	5-fold concentrated	55 ml	
3 pcs	microtiter plate + lid	-	-	
10 pcs	plate seals	-	-	

Sensitivity

MEAN calculated zero signal + 3 SD : 0.2 – 0.4 pg/ml (shake – static incubation)
 2x (MEAN calculated zero signal) : 0.5 – 1.0 pg/ml (shake – static incubation)

Expected values

IL-6 values in fresh serum and plasma samples of healthy individuals are below 20 pg/ml.

Specificity

No crossreactivity was observed with the following recombinant human proteins: IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor β -1 (TGF β -1), Tumour Necrosis Factor α (TNF- α), Tumour Necrosis Factor β (TNF β /Lymphotoxin), and Interferon γ (IFN γ).

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Standard

A recombinant huIL-6 standard has been calibrated against the WHO First International Standard (IL-6 89/548; National Institute for Biological Standards and Control, Potter Bar, Hertfordshire, U.K. 1 WHO Unit = 10 pg IL-6, see ref [20].

The kit contains one black-capped vial with 4000 pg/ml recombinant huIL-6

Avoid repeated freeze-thawing of the standard, although experimental data have shown that up to 3 freeze-thaw cycles have no effect on the IL-6 levels of the standard.

Standard curve

Label 7 tubes, one tube for each dilution: 450, 150, 50, 16.7, 5.6, 1.9 and 0.6 pg/ml. Pipette 497 μ l of working-strength dilution buffer into the tube labelled 450 pg/ml and 400 μ l of working strength dilution buffer into the other tubes.

Transfer 63 μ l of the IL-6 standard (4000 pg/ml) into the first tube labelled 450 pg/ml, mix well and transfer 200 μ l of this dilution into the second tube labelled 150 pg/ml.

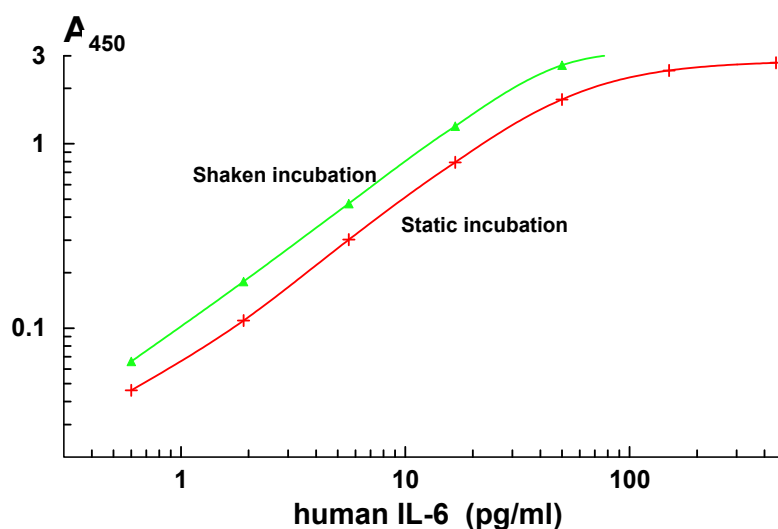
Repeat the serial dilutions five more times by adding 200 μ l of the previous tube of diluted standard to the 400 μ l of dilution buffer.

The standard curve will contain 450, 150, 50, 16.7, 5.6, 1.9, 0.6 and 0 pg/ml (dilution buffer).

It is recommended to prepare two separate series for each assay.

Samples

It is recommended to dilute the test samples at least 1:2 in working-strength dilution buffer. If high levels of IL-6 (outside the standard curve) are expected in the test samples, additional dilutions of sample i.e. 1:10 and 1:100 should also be prepared.

Typical standard curve


	STATIC INCUBATION	SHAKEN INCUBATION
	Calculated mean absorbance at 450 nm	
substrate blank	0	0
0 pg/ml	0.014	0.019
0.6 pg/ml	0.046	0.066
1.9 pg/ml	0.110	0.179
5.6 pg/ml	0.302	0.474
16.7 pg/ml	0.793	1.245
50 pg/ml	1.738	2.667
150 pg/ml	2.497	> 3.000
450 pg/ml	2.750	> 3.000

DO NOT USE THESE DATA TO CONSTRUCT A STANDARD CURVE FOR SAMPLE VALUE CALCULATIONS