PeliKine compact™ human IL-6

Sanquin

Specification sheet

REF 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 (hulL-6) in plasma and other body fluids, as well as in cell-culture supernatant.

Assay procedure

See Assay procedure for PeliKine™ compact ELISA kit.

Kitcomponent 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	750µl	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-1α, IL-1ß, 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 ß-1 (TGFß-1), Tumour Necrosis Factor α (TNF-α), Tumour Factor ß (TNFß/Lymphotoxin), and Interferon γ (IFNγ).

References

1. Gauldie,J. *et al* (1987) Proc.Natl.Acad.Sci.(USA) 84: 7251
2. Le,J. and Vilcek,J. (1989) Lab.Invest. 61: 588
3. Heinrich,P.C. *et al* (1990) Biochem.J. 265: 621
4. Kishimoto,T. (1989) Blood 74: 1
5. Helle,M. *et al* (1988) Eur.J.Immunol. 18: 957
6. Houssiau,F.A. *et al* (1988) Arthritis Rheum. 31: 784
7. Swaak,A.J.G. *et al* (1988) Scand.J.Rheumatol. 17: 469
8. Waage,A. *et al* (1989) Clin.Immunol.Pathol. 50: 394
9. Nijsten,M.W.N. *et al* (1988) Lancet 11: 921
10. Guo,Y. *et al* (1990) Clin.Immunol.Pathol. 54: 361
11. Van Oers,M.H.J. *et al* (1988) Clin.Exp.Immunol. 71: 314
12. Yoshimura,N. *et al* (1991) Transplantation 51: 172
13. Hack,C.E. *et al* (1989) Blood 74: 1704
14. Ludwig,H. *et al* (1991) Blood 77: 2794
15. Sheron,N. *et al* (1991) Clin.Exp.Immunol. 84: 449
16. Aarden,L.A. *et al* (1985) Lymphokines 10: 175
17. Van Snick,J. *et al* (1987) J.Exp.Med 165: 641
18. Helle,M. *et al* (1988) Eur.J.Immunol. 18: 1535
19. Helle,M. *et al* (1991) J.Immunol.Methods 138: 47
20. Gaines Das,R.E. *et al* (1993) J.Immunol.Methods 160: 147

Reagents
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands
Phone: +31 20 512 3599
Fax: +31 20 512 3570
E-mail: reagents@sanquin.nl
Website: www.sanquinreagents.com

p.t.o.

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 µl of working-strength dilution buffer into the tube labelled 450 pg/ml and 400 µl of workingstrength dilution buffer into the other tubes.
Transfer 63 µl of the IL-6 standard (4000 pg/ml) into the first tube labelled 450 pg/ml, mix well and transfer 200 µl of this dilution into the second tube labelled 150 pg/ml.
Repeat the serial dilutions five more times by adding 200 µl of the previous tube of diluted standard to the 400 µl of dilution buffer.

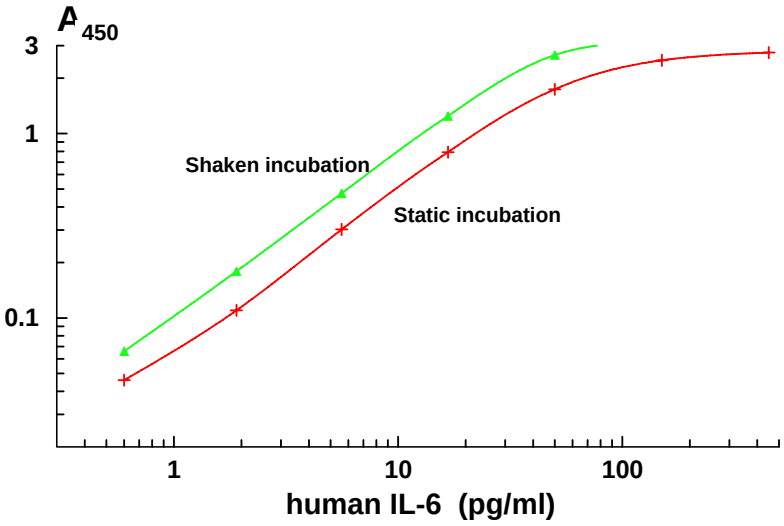
The standard curve will contain 450, 150, 50, 16.7, 5.6, 1.9, 0.6 and 0 pg/ml (dilution buffer).

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