Sanquin Reagents
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

Phone: +31 20 5123599
Fax: +31 20 5123570
reagents@sanquin.nl
www.sanquinreagents.com

PeliKine compact™ human IL1ß

Product number

M1934

Introduction

Interleukin 1 (IL-1) refers to two polypeptide hormones, interleukin-1α (IL-1α) and interleukin-1ß (IL-1ß), that possess a wide spectrum of inflammatory, metabolic, physiological, haematopoietic and immunological activities. Although the two forms of IL-1 are distinct gene products, they recognize the same receptor and share biological properties. Several substances originally described for their biological activities have been identified as IL-1; catabolin, endogenous pyrogen (EP), osteoclast-activating factor (OAF), epidermal cell-derived thymocyte-activating factor (ETAF), serum amyloid A inducer or hepatocyte-stimulating factor (HSF), leukocyte endogenous mediator (LEM), fibroblast-activating factor (FAF), B-cell-activating factor (BAF), proteolysis-inducing factor (PIF), haemopoeitin-1 (H-1), mononuclear cell factor (MCF), lymphocyte proliferation promoting factor of neutrophils, melanoma growth inhibition factor and tumour inhibitory factor 2.
The biological properties of IL-1 shows considerable overlap with other cytokines, including tumour necrosis factor (TNF) and interleukin 6 (IL-6). They all share the ability to stimulate T and B lymphocytes, to augment cell proliferation and to initiate or suppress gene expression for several proteins. Elevated levels of circulating IL-1 have been reported in patients with renal allograft rejection, acute attacks of rheumatoid arthritis, alcoholic hepatitis and burns. In patients undergoing routine haemodialysis, several studies have shown elevations in plasma IL-1 levels 3-4 hours following the initiation of the procedure. IL-1 has also been detected in synovial fluid of patients with rheumatic arthritis and in cerebrospinal fluid of patients with closed head trauma and meningitis. In addition elevated IL-1 levels were found in peritoneal, gingival, middle ear, ocular and nasal fluids of patients with various disease conditions.
Bioassays for the quantification of IL-1α or IL-1ß, based on the induction of IL-2 production by T-cell lines or proliferation of T-cell lines have been used for several years. These assays, although sensitive, are time consuming and might be susceptible to interference by other substances.
This PeliKine compact™ IL-1ß ELISA has been developed for faster, more reproducible and specific quantification of hun IL-1ß (huIL-1ß) in serum and 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 vial	IL-1ß standard (lyophilized)	see label	500 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 : 0.8 – 1.5 pg/ml (shake – static incubation)
2x (MEAN calculated zero signal) : 2.5 – 4.0 pg/ml (shake – static incubation)

Expected values

IL-1ß values in fresh serum and plasma of healthy individuals are below 5 pg/ml.

Specificity

No crossreactivity was observed with the following recombinant human proteins: IL-1α, IL-2,
IL-3, IL-4, IL-5, IL-6, sIL-6r (GP80), IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, Macrophage Colony Stimulating Factor (M-CSF), Gr Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukemia Inhibitory Fac RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor ß-1 (TGFß-1), Tumour Necrosis Fact (TNFα), Tumour Necrosis Factor ß (TNFß/Lymphotoxin), and Interferon γ (IFNγ).

References

1. Aggerwal,B.B. Gutterman,J.U. (1992) Human cytokines. Blackwell Sci.Pub. ISBN 0-86542-183-8
2. Ben-Aribia,M.H. *et al* (1987) J.Immunol. 139: 443
3. Bodel,P.T. *et al* (1968) Br.J.Exp.Pathol. 49: 11
4. Clowes,G.H.A. *et al* (1983) N.Engl.J.Med. 308: 545
5. Dinarello,C.A. *et al* (1986) J.Clin.Invest. 77: 1734
6. Dinarello,C.A. (1989) Adv.Immunol. 44: 153
7. Eastgate,J.A. *et al* (1988) Lancet 2: 706
8. Eisenberg,J.A. *et al* (1990) Nature 343:341
9. Falkoff,F.J. *et al* (1984) J.Immunol. 133: 443
10. Gahring,L.C. (1984) Fed.Proc. 43: 462
11. Gery,L. *et al* (1972) J.Exp.Med. 136: 128
12. Gray,P.W. *et al* (1986) J.Immunol. 137: 3644
13. Hannum,C.H. *et al* (1990) Nature 343: 336
14. asin,H.E. Dingle,J.T. (1981) J.Clin.Invest. 68: 571
15. March,C.J. *et al* (1985) Nature 315: 641
16. Oppenheim,J.J. *et al* (1986) Immunol.Today 7: 45
17. Porat, R. *et al* (1991) Science 254: 430
18. Thomson,A.W. (1991) The cytokine handbook. Academic Press ISBN 0-12-689660-7

Standard

A recombinant human IL-1ß standard has been calibrated against the WHO International Reference Preparation (IL-1ß 86/680; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 10 pg IL-1ß).

The kit contains one lyophilized vials with natural human IL-1ß.

Reconstitute the lyophilized standard by adding 500 µl of distilled water to the vial. Incubate for 10 minutes at room temperature and mix gently. After reconstitution the standard must be kept cold (2-8°C) and stored frozen after use (<-18°C, preferably<-70°C).

Standard curve

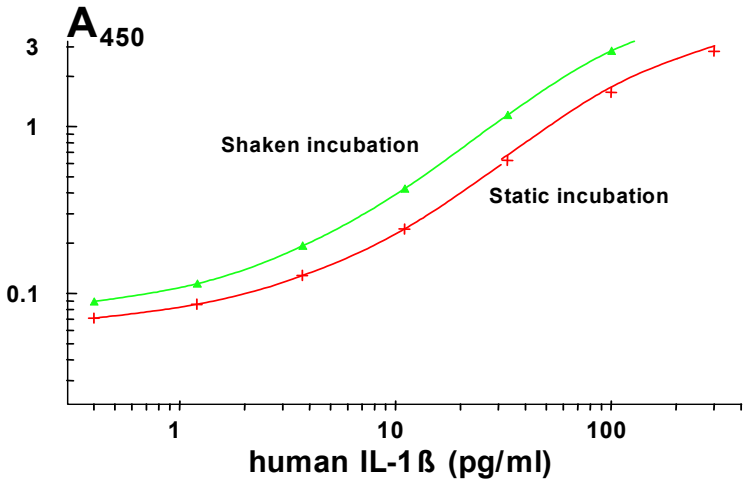
Label 7 tubes, one tube for each dilution: 300, 100, 33, 11, 3.6, 1.2 and 0.4 pg/ml.
Pipette 400 µl of working-strength dilution buffer into the tube labelled 300 pg/ml and 300 µl of working-strength dilution buffer into the other tubes.
Transfer 60 µl of the IL-1ß standard (2300 pg/ml) into the first tube labelled 300 pg/ml, mix well and transfer 150 µl of this dilution into the second tube labelled 100 pg/ml.
Repeat the serial dilution's six more times by adding 150 µl of the previous tube of diluted standard to the 300 µl of dilution buffer. The standard curve will contain 300, 100, 33, 11, 3.6, 1.2, 0.4 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-1ß (outside the standard curve) are expected in the test samples, additional dilutions of sample i.e. 1:10 and 1:50 should also be prepared (*If rheumatoid factors are expected in serum or plasma samples, it is recommended to add normal mouse serum (Sanquin product M1250, final concentration in the diluted sample should be 5%).*

Typical standard curve



	STATIC INCUBATION	SHAKEN INCUBATION
	Calculated mean absorbance at 450 nm	
substrate blank	0	0
0 pg/ml	0.025	0.024
0.4 pg/ml	0.076	0.029
1.2 pg/ml	0.041	0.052
3.7 pg/ml	0.083	0.125
11 pg/ml	0.198	0.340
33 pg/ml	0.582	1.033
100 pg/ml	1.558	2.582
300 pg/ml	2.777	> 3.000

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