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 IL-8

Product number

M1918

Introduction

Interleukin 8 (IL-8) is a cytokine with multifunctional actions. Several substances originally described for their biological activities have been identified as IL-8; monocyte-derived neutrophil chemotactic factor (MDNCF), neutrophil-activating peptide (NAP), neutrophil-activating factor (NAF), granulocyte chemotactic protein (GCP), T-lymphocyte chemotactic factor (TCF), leukocyte adhesion inhibitor (LAI). IL-8 was isolated first from stimulated leukocytes, but the molecule can be produced by a wide variety of cell types in response to cytokine inducers. In view of the increasing number of newly discovered chemotactic proteins, that are structurally related to IL-8, these cytokines are now designated 'chemokines'. The hallmark for this family of proinflammatory proteins is the conservation of four cysteine residues that are important for the tertiary structure. The chemokines can be devided into two subfamilies depending on whether the first two cysteines are adjacent (C-C chemokines) or not (C-X-C chemokines). IL-8 is a member of the second subfamily.
Elevated levels of IL-8 have been after sublethal endotoxaemia, septic shock, microbial infection of the amniotic cavity, Jarish-Herxheimer reaction of relapsing fever, infectious diseases of the central nervous system, acute pancreatitis, ulcerative colitis, empyaema, haemolitic uraemic syndrome, meningoccocal disease, gastric infection, pertussis, and peritonitis.
Bioassays for the quantification of IL-8, based on the ability to chemoattract neutrophils in vitro, have been used for several years. These assays, although sensitive, are time consuming and susceptible to interference by other substances.
This PeliKine compact™ human IL-8 kit has been developed for faster, more repro ducible and specific quantification of human (huIL-8) 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-8 standard	see label	200 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 : 1 – 3 pg/ml (shake – static incubation)
2x (MEAN calculated zero signal) : 4 – 8 pg/ml (shake – static incubation)

Expected values

IL-8 values in fresh serum and plasma samples of healthy individuals are below 10 pg/ml.

Specificity

No crossreactivity was observed with the following recombinant human proteins: IL-1 α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukaemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor β-1 (TGFβ-1), Tumour Necrosis Factor α (TNFα), Tumour Necrosis Factor β (TNFβ/Lymphotoxin), and Interferon γ (IFNγ).

References

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Standard

A natural huIL-8 standard has been calibrated against the WHO Interim International Standard (IL-8 89/520; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 10 ng IL-8). In former CLB IL-8 reagents sets [Batch IL8-CK0001 to IL8-CK0004 and 1918-00-05] 1 pg IL-8 standard is comparable with 0.56 pg of the WHO standard).

The kit contains one black-capped vial with 10 ng/ml natural IL-8

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-8 levels of the standard.

Standard curve

Label 7 tubes, one tube for each dilutions: 240, 96, 38.4, 15.4, 6.1, 2.5 and 1 pg/ml.
Pipette 610 µl of working-strength dilution buffer into the tube labelled 240 pg/ml and 300 µl of working-strength dilution buffer into the other tubes.
Transfer 15 µl of the IL-8 standard (10 ng/ml) into the first tube labelled 240 pg/ml, mix well and transfer 200 µl of this dilution into the second tube labelled 96 pg/ml.
Repeat the serial dilutions six more times by adding 200 µl of the previous tube of diluted standard to the 300 µl of dilution buffer.

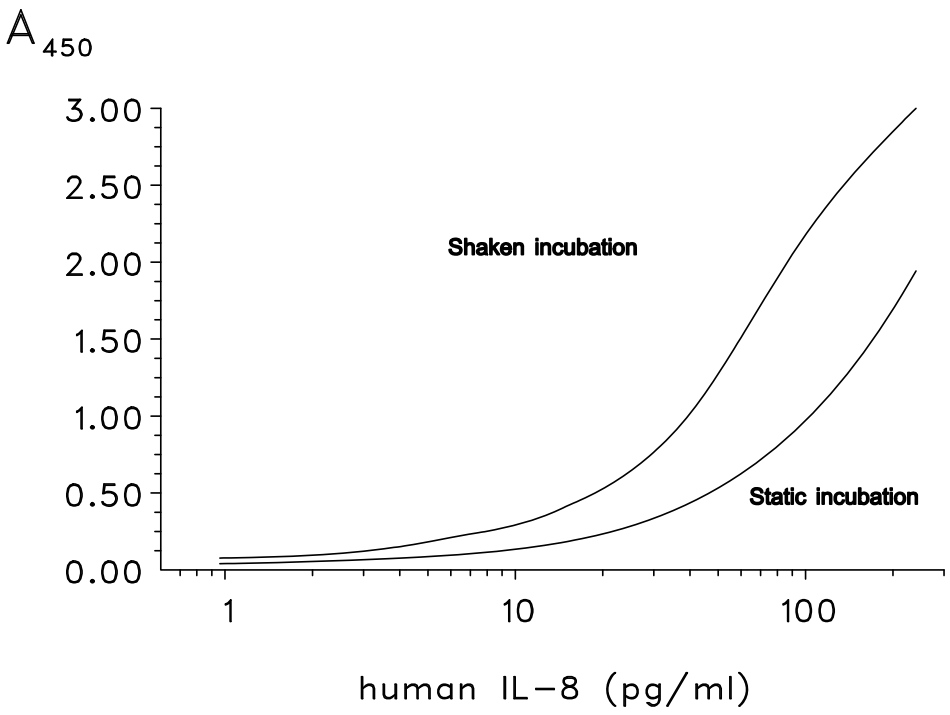
The standard curve will contain 240, 96, 38.4, 15.4, 6.1, 2.5, 1 and 0 pg/ml (dilution buffer).

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-8 (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.

Typical standard curve



	STATIC INCUBATION	SHAKEN INCUBATION
	Calculated mean absorbance at 450 nm	
substrate blank	0	0
0 pg/ml	0.038	0.041
1 pg/ml	0.041	0.077
2.5 pg/ml	0.060	0.107
6.1 pg/ml	0.097	0.215
15.4 pg/ml	0.189	0.424
38.4 pg/ml	0.418	0.974
96 pg/ml	0.940	2.130
240 pg/ml	1.943	3.000

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