PeliKine compact™ human IL-10

Sanquin

Specification sheet

REF M1910

Introduction

The initial discovery that led to the characterization and cDNA cloning of Interleukin 10 (IL-10) was the demonstration that supernatants from activated T-cells could inhibit the secretion of cytokines by TH1-cell clones [1,2]. Later it was found that IL-10, also known as cytokine synthesis inhibitory factor (CSIF) is an immunoregulatory protein produced by a number of cell lineages, like TH2-cells, B-cells and activated monocytes [1,3-8]. As an immune system down-regulator, IL-10 inhibits the synthesis of several cytokines that are normally secreted by both human and mouse monocytes/macrophages in response to activation by LPS. These cytokines include IL-1, GM-CSF, TNF, IL-6, IL-8, IL-10 and IL-12 [1,6,8]. IL-10 is secreted by activated macrophages, and this is also inhibited by IL-10 [6]. The secretion of IL-10 is relatively late compared with other cytokines, which may explain why macrophages are able to secrete substantial amounts of various cytokines before IL-10 inhibition occurs. IFNγ also inhibits macrophage secretion of IL-10 [9], resulting in direct cross-inhibition of IL-10 and IFNγ.
Nevertheless, IL-10 has also been shown to have up-regulating capacities, including the enhancement of *in vitro* proliferation of IL-2 and/or IL-4 induced mouse thymocytes, the induction of differentiation and proliferation of activated B-cell lines into antibody secreting lines [11], supporting the growth of mast cell lines [12] and induction of class II MHC antigen expression on B-cells [11,12].
Bioassays for the quantification of IL-10, based on the inhibition of IFNγ and other cytokines produced by TH1-cells in response to stimulation by antigen have been used for several years. These bioassays, although sensitive, are time consuming and susceptible to interference by other substances.
The Pelikine compact™ human IL-10 ELISA kit [10] has been developed for faster, more reproducible and specific quantification of human IL-10 in serum, 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
2 vials	IL-10 standard (lyophilized)	see label	1000 µl	-
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) : 3 – 5 pg/ml (shake – static incubation)

Expected values

IL-10 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-1ß, IL-2, 4, IL-5, IL-6, sIL-6r (GP80), IL-7, IL-8, IL-9, IL-11, IL-13, Macrophage Colony Stimulating Factor (M-Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor 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 (TNFα), Tumour Necrosis Fac (TNFß/Lymphotoxin), and Interferon γ (IFNγ).

References

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Standard

A recombinant human IL-10 standard has been calibrated against the WHO International Reference Preparation (IL-10 92/516; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 200 pg IL-10).

The kit contains two lyophilized vials with natural human IL-10.

Reconstitute the lyophilized standard by adding 1000 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, 120, 48, 19.2, 7.7, 3.1 and 1.2 pg/ml.
Pipette 470 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 30 µl of the IL-10 standard (5000 pg/ml) into the first tube labelled 300 pg/ml, mix well and transfer 200 µl of this dilution into the second tube labelled 120 pg/ml.
Repeat the serial dilution's six more times by adding 200 µl of the previous tube of diluted standard to the 300 µl of dilution buffer.

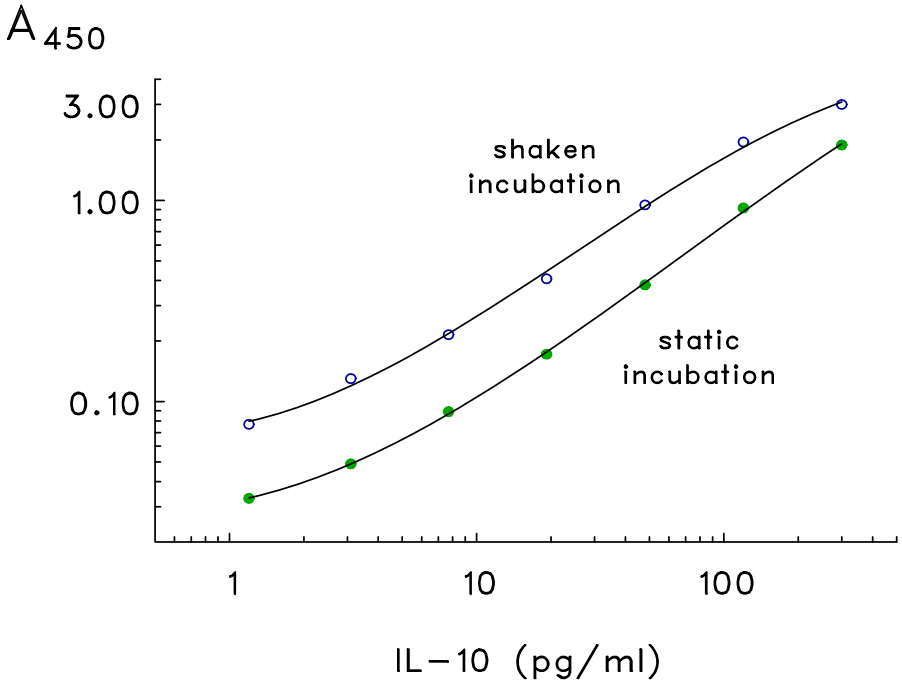
The standard curve will contain 300, 120, 48, 19.2, 7.7, 3.1, 1.2 and 0 pg/ml (dilution buffer).

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-10 (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	0.021	0.067
1.2 pg/ml	0.033	0.077
3.1 pg/ml	0.049	0.178
7.7 pg/ml	0.089	0.215
19.2 pg/ml	0.172	0.408
48 pg/ml	0.380	0.950
120 pg/ml	0.917	2.259
300 pg/ml	1.886	> 3.000

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