PeliKine compact™ human TNFα

Sanquin

Specification sheet

REF M1923

Introduction

Tumour necrosis factor α (TNFα) is an extremely potent peptide cytokine which serves as an endogenous mediator of inflammatory, immune and host defence functions. Several substances originally described for their biological activities have been identified as TNFα; cachectin, macrophage cytotoxin (MCT), necrosin, cytotoxin (CTX), haemorrhagic factor, macrophage cytotoxic factor (MCF) and differentiation-inducing factor (DIF).
TNFα is capable of acting independently and in conjunction with a variety of other factors to affect the phenotype and metabolism of cells in every tissue of the body. It is generally thought that TNFα is not produced constitutively by normal cells, but rather to be induced potently by invasive stimuli in the setting of both neoplastic and infectious disease. In this role, macrophages and monocytes are thought to be the cells which contribute most to the local and systemic TNFα response to bacterial, viral and parasitic organisms and products.
Bioassays for the quantification of TNFα, including the cytotoxic assay on murine fibroblasts have been used for several years. However, TNFα shares many of the biological effects of IL-1 and for this reason the two commonly interfere in bioassays. Although the cytotoxic assay mentioned above, is unaffected by IL-1, it remains time consuming and might be susceptible to interference by other substances.
The Pelikine compact™ human TNFα ELISA kit has been developed for faster, more reproducible and specific quantification of human TNFα 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
1 vials	TNFα 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 : 1 – 3 pg/ml (shake – static incubation)
2x (MEAN calculated zero signal) : 4 – 6 pg/ml (shake – static incubation)

Expected values

TNFα 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-1α, IL-1ß, 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), 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 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. Ammann,A.J. *et al* (1987) J.Clin.Immunol. 7: 6
3. Beutler,B. *et al* (1985) Science 229: 869
4. Beutler,B. *et al* (1985) Nature 316: 552
5. Carswell,E.A. *et al* (1975) Proc.Natl.Acad.Sci. 72: 3666
6. Dinarello,C.A. *et al* (1986) J.Exp.Med. 163: 1433
7. Dinarello,C.A. *et al* (1988) Rev.Inf.Diseas. 10: 168
8. Exely, A.R. *et al* (1990) Cytokine 2: 353
9. Kwiakowski,D.A. *et al* (1990) The Lancet 336: 1201
10. Pennica,D. *et al* (1984) Nature 312: 724
11. Silberstein,S.B. *et al* (1986) Proc.Natl.Acad.Sci. 83: 1055
12. Sugarman,B.J. *et al* (1985) Science 230: 943
13. Thomson,A.W. (1991) The cytokine handbook. Academic Press ISBN 0-12-689660-7
14. Tori,F.M. *et al* (1985) Science 229: 867
15. Tracey,K.J. *et al* (1986) Science 23: 470
16. Wong,G.H.W. *et al* (1986) Nature 323: 819

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 natural human TNFαstandard has been calibrated against the WHO International Standard (TNFα 87/650; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 25 pg TNFα).

The kit contains one lyophilized vial with natural TNFα.

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

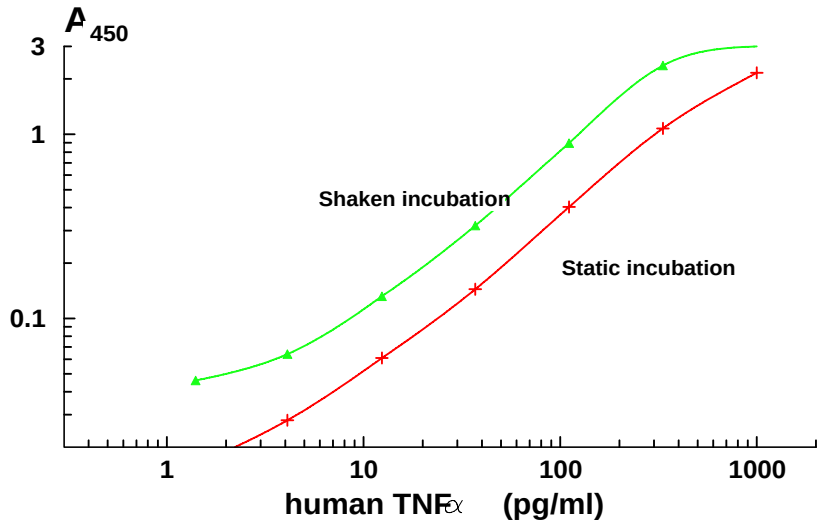
Label 7 tubes, one tube for each dilutions: 1000, 333, 111, 37, 12.4, 4.1 and 1.4 pg/ml.
Pipette 168 µl of working-strength dilution buffer into the tube labelled 1000 pg/ml and 150 µl of workingstrength dilution buffer into the other tubes.
Transfer 70 µl of the TNFα standard (3400 pg/ml) into the first tube labelled 1000 pg/ml, mix well and transfer 75 µl of this dilution into the second tube labelled 333 pg/ml.
Repeat the serial dilutions five more times by adding 75 µl of the previous tube of diluted standard to the 150 µl of dilution buffer.
The standard curve will contain 1000, 333, 111, 37, 12.4, 4.1, 1.4 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 TNFα (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 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	0.012	0.029
1.4 pg/ml	0.016	0.046
4.1 pg/ml	0.028	0.064
12.4 pg/ml	0.061	0.132
37 pg/ml	0.144	0.391
111 pg/ml	0.403	0.895
333 pg/ml	1.076	2.361
1000 pg/ml	2.157	≥ 3.000

DO NOT USE THESE DATA TO CONSTRUCT A STANDARD CURVE FOR