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PeliKine compact™ human IFN-gamma

Product number

M1933

Introduction

At this moment fifteen interferon α (IFNα), one interferon β (IFNβ) and one interferon γ (IFNγ) have been reported. IFNγ is produced during an immune response by $CD8^+$, NK, γδ and TH1 T helper cells. It differs structurally and functionally from IFNα and IFNß; binds to distinct receptors and has pronounced immuno-regulatory effects, including activation of macrophages to enhance phagocytosis and tumour killing capability, activation and growth enhancement of cytolytic T-cells and NK-cells, and induction of class II MHC antigen and Fcγ receptor on macrophages and many other cell types.
IFNγ also regulates humeral immune responses: it induces immunoglobulin secretion by activated B-cells stimulated with IL-2 and potentiates IL-4 induced proliferation of human B-cells. IFNγ has documented antiviral and antiprotozoal activities, although IFNα and IFNß seem to have more potent antiviral activities than IFNγ.
Several substances originally described for their biological activities have been identified as IFNγ; macrophage activating factor (MAF), T-cell replacing factor (TRF), Type II interferon and immune interferon.
Bioassays for the quantification of IFNγ, based on cytopatic reductive effects of IFNγ on cultured cells have been used for several years. In this assay IFNγ reduces the killing of a target cell line such as L929 (murine), HEp2C or A549 (human) cells by for example, encephalomyocarditis virus. An alternative assay system involves measurement of induction of HLA-DR antigens on tumour cells, which can be detected in a cell ELISA. These assays, although sensitive, are time consuming and might be susceptible to interference by other substances.
The Pelikine (compact)™ human IFNγ ELISA kit has been developed for faster, more reproducible and specific quantification of human IFNγ in serum, 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
2 vials	IFNγ 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 : 1-2 pg/ml (shake - static incubation)
2 x (MEAN calculated zero signal) : 4-6 pg/ml (shake - static incubation)

Expected values

IFNγ 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-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, 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) and Tumour Necrosis Factor β (TNFβ/Lymphotoxin).

References

1. Adolf,G.R. (1985) Oncology 42: 33
2. Balkwill,F. (1989) The Lancet (I): 1060
3. Billiau,A. (1988) Immunology Today 9: 37
4. Billiau,A. *et al* (1990) Biochem.Pharmacol. 40: 1433
5. Bruserud,O. *et al* (1993) Eur.J.Hematol. 51: 73
6. Celada,A. *et al* (1989) Eur.J.Immunol. 19: 1103
7. Doldi,K. *et al* (1985) J.Interferon Res. 5: 55
8. Farrar,M.A. *et al* (1993) Ann.Rev.Immunol. 11: 571
9. Gray,P.W. *et al* (1892) Nature 295: 503
10. Grossberg, S.E. *et al* (1989) Experientia 45: 508
11. Ijzermans,J.M. *et al* (1989) Immunobiol. 179: 456
12. Kwiatkowski,D.A. *et al* (1990) The Lancet 336: 1201
13. Locksley, R.M. *et al* (1991) Immunoparasitology Today: A58
14. Mogensen, S.C. *et al* (1987) Interferon 8: 55
15. O'Garra,A. (1989) The Lancet (I): 1003
16. Paliard,X. (1988) *et al* J.Immunol. 141: 849
17. Reiter,Z. (1993) J.Interferon Res. 13: 247
18. Samuel, C.E. (1991) Virology 183: 1
19. Thomson,A.W. (1991) The cytokine handbook. Academic Press ISBN 0-12-689660-7

Standard

A natural human IFNγ standard has been calibrated against the WHO reference preparation (IFNγ 88/606; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 53 pg IFNγ).
The kit contains one lyophilized vial natural human IFNγ
Reconstitute one 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

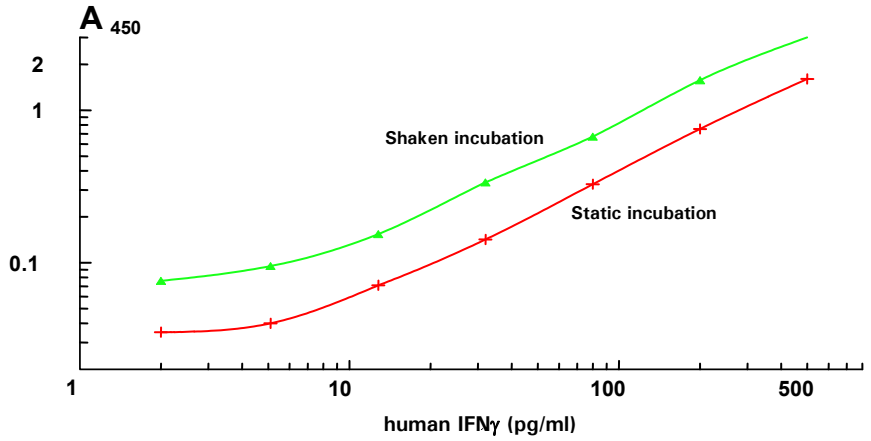
Label 7 tubes, one tube for each dilution: 500, 200, 80, 32, 12.8, 5.1, and 2.0 pg/ml. Pipette 600 µl of working-strength HPE-dilution buffer into the tube labelled 500 pg/ml and 300 µl of working-strenght HPE-dilution buffer into the other tubes. Transfer 75 µl of the IFNγ standard (4500 pg/ml) into the first tube labelled 500 pg/ml, mix well and transfer 200 µl of this dilution into the second tube labelled 200 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. Label one tube 0 pg/ml (working-strength HPE-dilutionbuffer)

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 HPE-dilution buffer. If high levels of IFNγ (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	0.025	0.073
2.0 pg/ml	0.035	0.074
5.1 pg/ml	0.040	0.095
12.8 pg/ml	0.071	0.154
32 pg/ml	0.142	0.336
80 pg/ml	0.327	0.671
200 pg/ml	0.753	1.572
500 pg/ml	1.604	> 3.000

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