Fast Human IFN-Gamma ELISA Maxi

For the quantitation of human IFN-γ concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

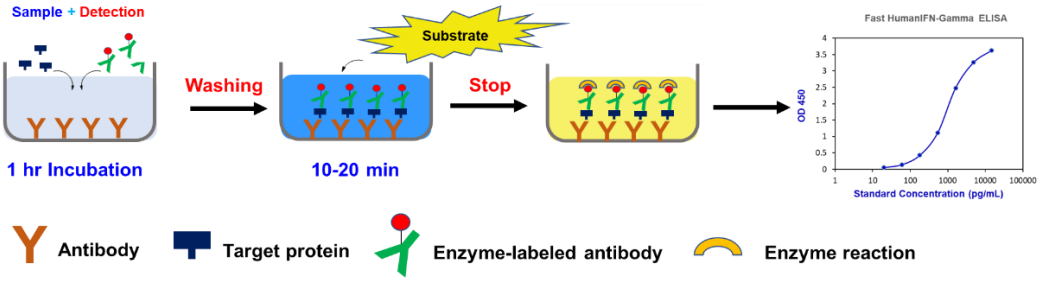
Interferon-gamma (IFN-γ, also known as type II interferon) is an important immunoregulatory cytokine through its anti-viral activity. IFN- γ is produced by a number of cell types under inflammatory conditions, including dendritic epidermal cells, T cells, keratinocytes, peripheral blood T cells, mast cells, neurons, CD8+T cells, macrophages, B cells, neutrophils, NK cells, CD4+T cells, and testicular spermatids. It plays key roles in host defense, and the progression of inflammatory diseases such as autoimmunity and atherosclerosis by exerting anti-viral, anti-proliferative, and immunoregulatory activities.

The Tribio® Fast Human IFN- γ ELISA Maxi is a solid phase ELISA designed to measure human IFN- γ levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detection into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour instead of 5-6 hours (Fig. 1). The detection range is from 20 to 15000 pg/mL. The levels of human IFN- γ samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human IFN- γ protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IFN- γ was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then, incubated with HRP-conjugated detection antibody specific for human IFN- γ. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of IFN- γ bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig.1 Novel Proprietary Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE CONDITIONS
Human IFN- γ Microplate	TBS3230A	96 well strip microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human IFN- γ.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human IFN- γ Standard	TBS3230B	100 µL of Recombinant human IFN- γ protein (145.8ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3230C	2.2 ml of HRP-Human IFN- γ antibody.	May be stored for up to 3 months at 2-8 °C.*
Assay Diluent	TBS3230D	12 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

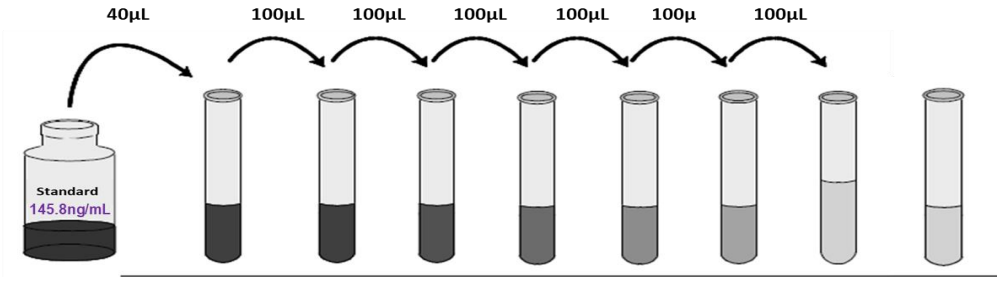
Human IFN-γ Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below (Fig2.).**

2. Add 40 µL of the Human IFN- γ Standard stock solution (145.8ng/mL) by dilution of 10 times to tube #1 and mix completely.

3. Take 100 µL of the Human IFN- γ standard from tube #1 to tube #2 and mix completely. Repeat 3 x serial dilutions for tubes #3 through #7. The standard concentration in tube 1 through 7 will be 14580, 4860, 1620, 540, 180, 60 and 20 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for IFN-Gamma Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Diluent (µL)	**360**	**200**	**200**	**200**	**200**	**200**	**200**	**200**
Addition	**Stock**	**Std1**	**Std2**	**Std3**	**Std4**	**Std5**	**Std6**	
Addition Vol. ((µL)	**40**	**100**	**100**	**100**	**100**	**100**	**100**	
Std Conc (pg/mL)	14580	4860	1620	540	180	60	20	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour.**
3. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at

540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate.

CALCULATION OF RESULTS

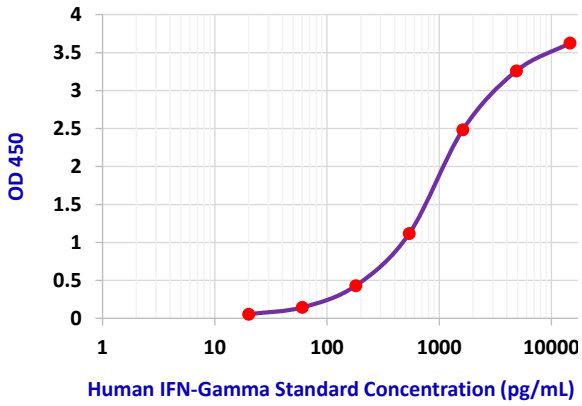
Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IFN-γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is provided for demonstration only as Fig.3. A standard curve should be generated for each set of samples assayed.

Fig.3 Fast Human IFN-Gamma Standard



SENSITIVITY

The minimum detectable dose (MOD) of human IFN-γ is typically 8 pg/ml.

SPECIFICITY

This assay recognizes natural and recombinant human IFN- γ.

REFERENCES

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3. Pestka,S. et al. (2004): lmmunol. Rev.202:8.
4. Kelchtermans,H. et al. (2008): Trends lmmunol. 29:479.
5. Mclaren,J.E. and D.P. Ramji (2009): Cytokine Growth Factor Rev.20:125.

RELATIVE PRODUCTS

Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (TBS5001)
Protein Assay Kit (TBS2005)
TMB Substrate System (TBS5021)

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