Fast Monkey IFN-gamma ELISA

For the quantitation of Monkey 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 in the progression of inflammatory diseases such as autoimmunity and atherosclerosis by exerting anti-viral, anti-proliferative, and immunoregulatory activities.

The Tribio® Fast Monkey IFN- γ ELISA kit is a solid phase ELISA designed to measure monkey IFN- γ levels in cell culture supernatants, serum, and plasma. The main feature of this kit is its use of a novel fast approach to replace the traditional methods. So that it makes the assay short procedures, and only one-step washing out. **The measurement can be finished within 1.5 hours instead of the traditional 4-5 hours**. **The detection range is between 20 and 15,000 pg/mL**. The levels of monkey 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 monkey IFN- γ protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique. A monoclonal antibody specific for monkey IFN- γ was pre-coated onto a microplate. Standards and samples are pipetted into the wells and incubated with HRP-conjugated detection antibody specific for Monkey IFN- γ. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells develop color. 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.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Monkey IFN- γ Microplate	TBS3231A	96-well polystyrene microplate (12-strips of 8-wells) coated with a polyclonal antibody specific for monkey IFN- γ.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Monkey IFN- γ Standard	TBS3231B	0.1mL of Recombinant monkey IFN-γ protein (145.8 ng/mL).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3231C	2.2ml of HRP-Monkey IFN- γ antibody.	May be stored for up to 3 months at 2-8 °C.*
Assay Diluent	TBS3231D	12ml of a buffered protein base with preservatives.	
Wash Buffer Concentrate	TBS3000W	12ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6ml of 2N sulfuric acid.	
Plate Sealers	N/A	Adhesive strips.	

Store the unopened kit at 2-8 ℃. 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-ware, and facial protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

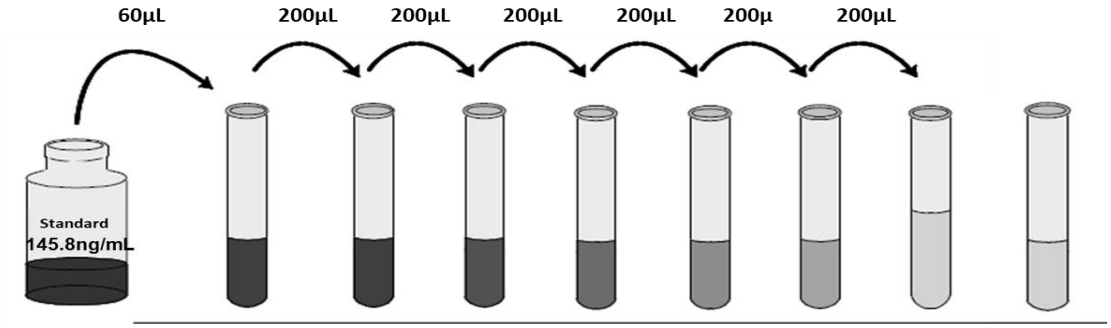
Bring all reagents to room temperature before use.

Wash Buffer: Add 10mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of 1x Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Monkey IFN-γ Standard Preparation:

1. Label test tubes 1-8. Pipet 540µL of 1x Assay Diluent into tube #1 and 200 µL into tubes #2 through #8 **as shown in the diagram below.**
2. Add 60 µL of the Monkey IFN- γ Standard stock solution (145.8ng/mL) to tube #1 for a 10x dilution. Mix thoroughly.
3. Take 200 µL of the Monkey IFN- γ standard from tube #1 and add to tube #2. Mix thoroughly. Repeat the 3x serial dilution for tubes #3 through #7. The standard concentration in tube 1-7 will be 14,580, 4,860, 1,620, 540, 180, 60 and 20 pg/mL respectively. Tube #8 is Standard 0.

Diagram for IFN-Gamma Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Diluent (µL)	540	200	200	200	200	200	200	200
Stock (145.8ng/mL)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
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 80µL of standard, sample, or control to each well.
2. Add 20µL of **Detection A** to the above standard and sample for each well. Mix thoroughly. Cover with the adhesive sealer. Incubate at **RT for 1 hour.**
3. Aspirate each well, and wash 3x by filling each well with 300µL of Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Shield from light*). A blue will develop.
5. Add 50µL 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 540nm or 570nm. 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. Readings made directly at 450 nm without correction may be higher and less accurate.

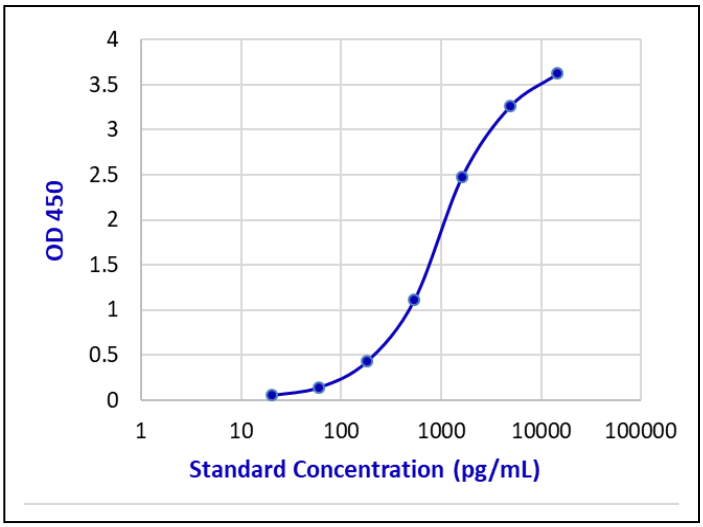
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then 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 Monkey 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. A standard curve should be generated for each set of samples assayed.



SENSITIVITY

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

SPECIFICITY

This assay recognizes natural and recombinant Monkey IFN- γ.

RELATIVE PRODUCTS

Human AFP ELISA (TBS3212)
Human HE4 ELISA (TBS3213)
Human CA125 ELISA (TBS3214)
Human CA19-9 ELISA (TBS3215)
Human CA15-3 ELISA (TBS3216)
Human PSA ELISA (TBS3217)
Human IFN-gamma ELISA (TBS3230)
Human TGF-beta1 ELISA (TBS3232)
Human ß2-microglobulin ELISA (TBS3218)
Protein Cell Lysis Buffer (TBS5001)
Protein Assay Kit (TBS2005)
Ultra-sensitive TMB Substrate System (TBS5021)
ADHP Fluorescent Substrate system (TBS5026)

For research use only. Not for use in diagnostic procedures.