Human IFN-ELISA Kit

User Manual

Kit Protocol

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I. INTRODUCTION

IFN-? is produced mainly by T-cells and natural killer cells activated by antigens, mitogens, or alloantigens. It is produced by lymphocytes expressing the surface antigens CD4 and CD8. IFN-? is a dimeric protein with subunits of 146 amino acids. The protein is glycosylated at two sites. The pI is 8.3-8.5. IFN-? inhibits the growth of B-cells induced by IL-4. IFN-? inhibits the proliferation of smooth muscle cells of the arterial intima in vitro and in vivo and therefore probably functions as an endogenous inhibitor for vascular overreactions such as stenosis following injuries of arteries.

The Human IFN-? ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IFN-? in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IFN-? coated on a 96-well plate. Standards and samples are pipetted into the wells and IFN-? present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IFN-? antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IFN-? bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. REAGENTS

1. IFN-? Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human IFN-?.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials, recombinant human IFN-?.

4. Assay Diluent A (Item D): 30 ml, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Detection Antibody IFN-? (Item F): 2 vial of biotinylated anti-human IFN-? (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 8 µl of 20,000x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

III. STORAGE

May be stored for up to 5 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at –80 °C) after reconstitution. Opened Microplate Wells and reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge of zip-seal.

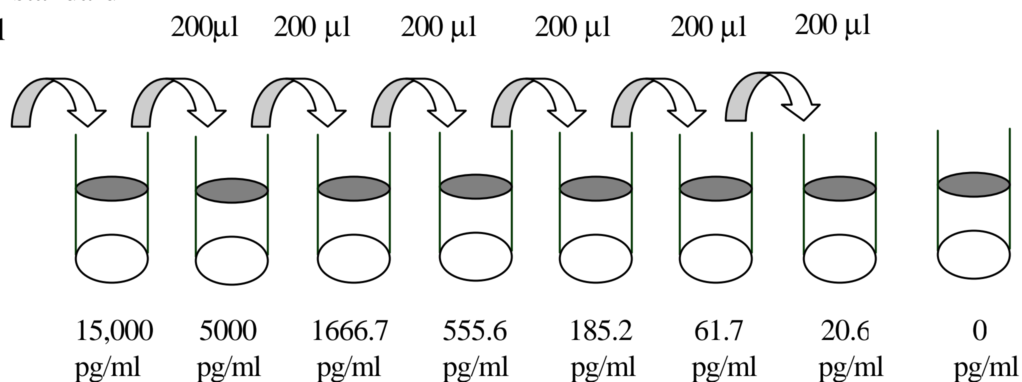
IV. ADDITIONAL MATERIALS REQUIRED

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 2 µl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Log-log graph paper or computer and software for ELISA data analysis.
8 Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix**. Add 180 µl IFN-? standard from the vial of Item C, into a tube with 420 µl Assay Diluent A or 1x Assay Diluent B to prepare a 15,000 pg/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



3. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants and urine.

4. Assay Diluent B should be diluted 5-fold with deionized or distilled water.

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 20,000-fold with 1x Assay Diluent B.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 60 µl of prepared 100-fold diluted solution into a tube with 12 ml 1x Assay Diluent B to prepare a final 20,000 fold diluted HRP-Streptavidin solution.

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

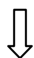
2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C.

3. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each).

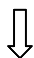
4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature.
5. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each).
6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature.
7. Discard the solution and wash 5 times with 1x Wash Solution (200 µl each).
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

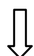
1. Prepare all reagents, samples and standards as instructed.



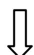
2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.



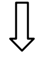
3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.



4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.



5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.



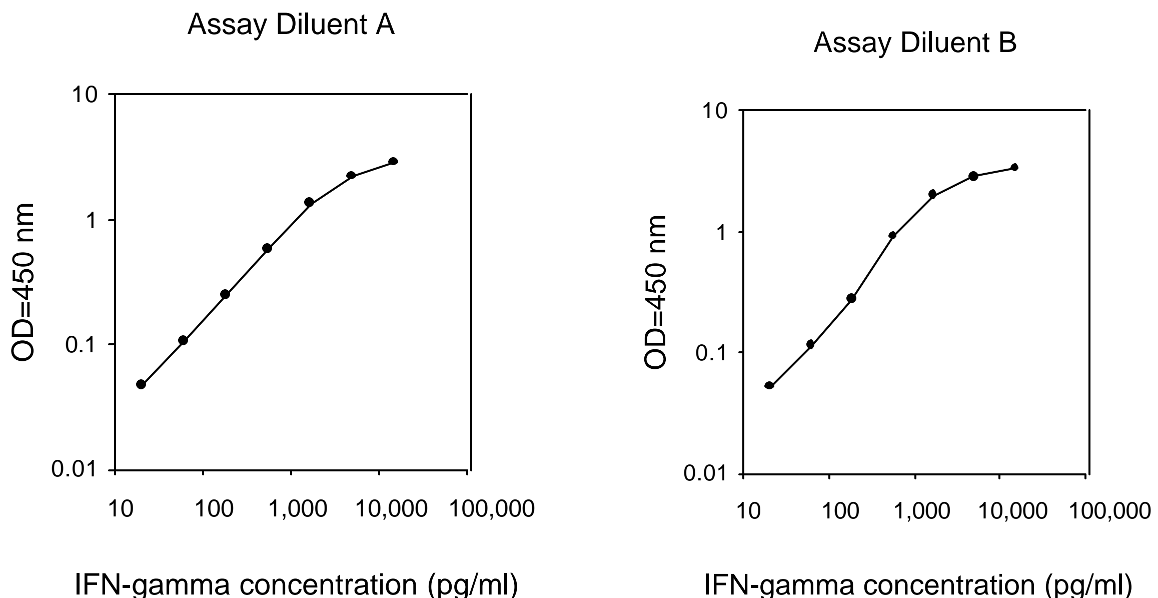
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of IFN-? is typically less than 15 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of human IFN-? into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	88.65	82-103
Plasma	86.82	81-102
Cell culture media	94.53	84-104

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	94	96	97
	Range (%)	80-99	82-102	83-103
1:4	Average % of Expected	95	97	95
	Range (%)	82-102	83-103	82-103
1:8	Average % of Expected	97	95	100
	Range (%)	84-103	83-104	86-106

E. REPRODUCIBILITY

Intra-Assay: CV<10%
Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (*e.g*., human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1d, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF).

X. REFERENCES:

1. De Maeyer E and De Maeyer-Guignard J Interferon-gamma. Current Opinion in Immunology 4: 321-6 (1992).

2. Dayton MA et al Human B cell lines express the interferon gamma gene Cytokine 4: 454-60 (1992).

3. Gu D and Sarvetnick N Epithelial cell proliferation and islet neogenesis in IFN-gamma transgenic mice. Development 118: 33-46 (1993).

XI. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at<-20°C after reconstitution, others at 4 °C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

Notes: