RayBio® Human IFN-gamma ELISA Kit (For Lysates)

Catalog #: ELH-IFNg-CL

User Manual
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Caution:
Extraordinarily useful information enclosed

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RayBio® Human IFN-gamma ELISA Kit (For Lysates) Protocol

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Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

IFN-gamma 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-gamma 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-gamma inhibits the growth of B-cells induced by IL-4. IFN-gamma 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 RayBio® Human IFN-gamma ELISA kit is an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IFN-gamma cell lysate and tissue lysate. This assay employs an antibody specific for human IFN-gamma coated on a 96-well plate. Standards and samples are pipetted into the wells and IFN-gamma present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IFN-gamma 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-gamma bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Need your results faster? Try RayBiotech's *SpeedE*LISA platform for quantitative detection in just three hours.
https://www.raybiotech.com/speedelisa-kits/

Short on sample, or need higher sensitivity? Check out the IQELISA™ Immuno-PCR platform.
https://www.raybiotech.com/immuno-pcr/

II. STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

III. REAGENTS

Component	Size / Description	Storage / Stability After Preparation
IFN-gamma Microplate (Item A)	96 wells (12 strips x 8 wells) coated with anti-Human IFN-gamma.	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Standard Protein (Item C)	2 vials of Human IFN-gamma. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
Detection Antibody IFN-gamma (Item F)	2 vials of biotinylated anti-Human IFN-gamma. Each vial is enough to assay half the microplate.	5 days at 4°C
HRP-Streptavidin Concentrate (Item G)	200 µl 400X concentrated HRP-conjugated streptavidin.	Do not store and reuse.
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A
Sample Diluent Buffer (Item D2)	10 ml of 5X concentrated buffer.	1 month at 4°C
Assay Diluent (Item E2)	15 ml of 5X concentrated buffer.	1 month at 4°C
Lysis Buffer (Item J)	5 ml of 2X cell lysate buffer.	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

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

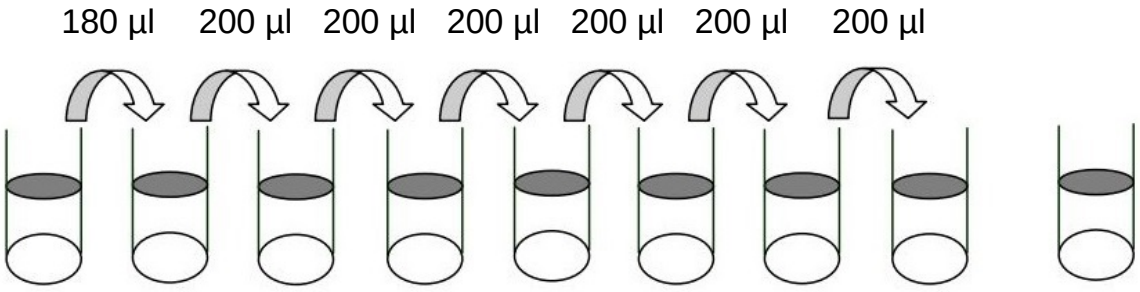
1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Sample Diluent Buffer (Item D2) and Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use. Lysis Buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

3. Sample dilution: Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1X Sample Diluent Buffer (Item D2). Generally we recommend a minimum of 1 mg of protein per 1 ml of original lysate solution, though more concentrated is better. We also recommend the addition of protease inhibitors (not included) to the lysis buffer prior to use. Detailed recommendations on lysis preparation may be found here: www.raybiotech.com/tips-on-sample-preparation.html

   Note: Levels of IFN-gamma may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl 1X Sample Diluent Buffer (Item D2) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 180 µl IFN-gamma standard from the vial of Item C, into a tube with 420 µl Sample Diluent Buffer to prepare a 15,000 pg/ml stock standard solution. Pipette 400 µl 1X Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Sample Diluent Buffer serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 400 µl	420 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	50 ng/ml	15000 pg/ml	5000 pg/ml	1666.7 pg/ml	555.6 pg/ml	185.2 pg/ml	61.7 pg/ml	20.6 pg/ml		0 pg/ml

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 (Item E2) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent (Item E2) and used in step 5 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, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 400-fold with 1X Assay Diluent (Item E2).

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 30 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1X Assay Diluent to prepare a 400-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

VI. ASSAY PROCEDURE

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

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. 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.

5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. 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.

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.

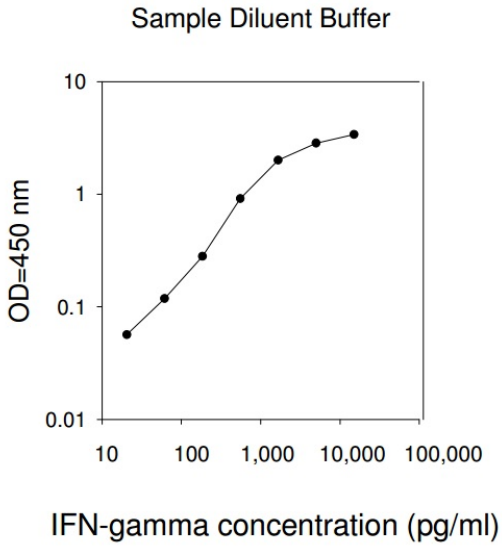
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

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 Human IFN-gamma was determined to be 15 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

C. SPIKING & RECOVERY

Recovery was determined by spiking various levels of Human IFN-gamma into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	91.73	80-101
Cell lysate	92.25	82-103

D. LINEARITY

Sample Type		Tissue Lysate	Cell lysate
1:2	Average % of Expected Range (%)	91 80-101	92 81-102
1:4	Average % of Expected Range (%)	96 84-103	95 83-104

E. REPRODUCIBILITY

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

IX. SPECIFICITY

The antibody pair provided in this kit recognizes human IFN-gamma.

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate

RayBio® ELISA Kits

Over 4,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits for details.

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