Human IFNγ ELISA Kit

EHIFNG EHIFNG2 EHIFNG5

Number	Description
EHIFNG	**Human Interferon gamma (IFNγ) ELISA,** sufficient reagents for 96 determinations
EHIFNG2	**Human Interferon gamma (IFNγ) ELISA,** sufficient reagents for 2 × 96 determinations
EHIFNG5	**Human Interferon gamma ELISA,** sufficient reagents for 5 × 96 determinations

Kit Contents	EHIFNG	EHIFNG2	EHIFNG5
Anti-human IFNγ Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human IFNγ Standard	2 vials	4 vials	10 vials
Standard Diluent, contains 0.1% sodium azide	12mL	2 × 12mL	5 × 12mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Biotinylated Antibody Reagent, contains 0.1% sodium azide	8mL	2 × 8mL	5 × 8mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	6 each	12 each	30 each

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

Storage: Upon receipt store the kit at 2-8°C.

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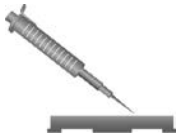
Introduction

The Invitrogen™ Human Interferon gamma (IFNγ) ELISA Kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of IFNγ in serum, plasma, urine and culture supernatant.

Procedure Summary

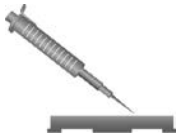


1. Add 50µL of Biotinylated Antibody Reagent to each well.

2. Add 50µL of Standards or samples to each well in duplicate.

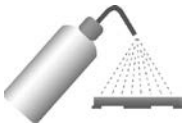
3. Cover plate and incubate at room temperature (20-25°C) for 2 hours.

4. Wash plate THREE times.



5. Add 100µL of prepared Streptavidin-HRP Solution to each well.

6. Cover plate and incubate at room temperature for 30 minutes.



7. Wash plate THREE times.

8. Add 100µL of TMB Substrate to each well.

9. Develop plate in the dark at room temperature for 30 minutes.

10. Stop reaction by adding 100µL of Stop Solution to each well.

11. Measure absorbance. Subtract 550nm values from 450nm values.

12. Calculate results using graph paper or curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- All samples and reagents must be at room temperature (20-25°C) before use in the assay.
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a water bath to thaw samples. Thaw samples at room temperature.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.
- To avoid cross-contamination always use a new disposable reagent reservoir and new disposable pipette tips for each transfer.
- Use a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.

- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Discard unused ELISA components after assay completion. Do not mix reagents from different kit lots.
- Do not use glass pipettes to measure TMB Substrate. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some kit components contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precautions for the 2-plate and 5-plate Kits

- Dispense, pool and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation

Sample Handling

- Serum; EDTA, heparin and sodium citrate plasma; urine; and culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.
- Samples and standards must be assayed in duplicate each time the ELISA is performed.

Sample Dilution

- If the IFNγ concentration possibly exceeds the highest point of the standard curve (i.e., 1000pg/mL), prepare one or more 10-fold dilutions of the sample. When testing **culture supernatants**, prepare serial dilutions using culture medium. When testing **serum, plasma or urine,** prepare serial dilutions using the Standard Diluent provided. A 10-fold dilution is prepared by adding 50µL of sample to 450µL of appropriate diluent. Mix thoroughly between dilutions.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout this instruction booklet.

Wash Buffer

Note: Wash buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

1. Label a clean glass or plastic two-liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. Add the entire contents of the 30X Wash Buffer (50mL) bottle to the container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

   (PP) When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

Standards

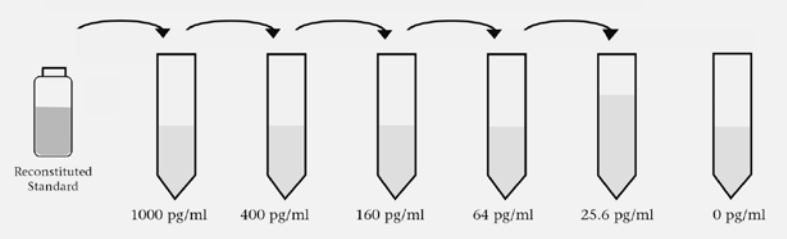
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

1. When testing **culture supernatant samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the vial label. The standard dissolves in approximately 1 minute. Mix by gently inverting vial. Use the sample culture medium to prepare dilutions of the standard.

   When testing **serum, plasma or urine samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the vial label. The standard dissolves in approximately 1 minute. Mix by gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

   When testing **serum, plasma or urine and cell culture supernatant samples on the same plate**, validate the media to establish if the same standard curve can be used for both sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Use medium-containing serum or other protein to maximize stability of the IFNγ. Perform this curve in parallel with a standard curve prepared with Standard Diluent. If OD values are within 10% of the mean for both curves, the assay may be performed with Standard Diluent, whether testing culture supernatant, urine, plasma or serum samples.
2. Label six tubes, one for each standard curve point: 1000pg/mL, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 240µL of appropriate diluent into each tube.
4. Pipette 160µL of the reconstituted standard into the first tube (i.e., 1000pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube labeled (i.e., 400pg/mL) and mix.
6. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

Serial dilutions using 160µL



Assay Procedure

A. Biotinylated Antibody Reagent and Sample Incubation

- **(PP)** Determine the number of strips required and leave these strips in the plate frame. Tightly seal unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
- Use the Data Template provided to record locations of the zero standard (blank or negative control), standards and samples. Perform three standard points and one blank in duplicate with each series of unknown samples.
- If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent. Remove from the vial only the reagent amount required for the number of strips being used. Take care not to touch samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.

1. Add 50µL of Biotinylated Antibody Reagent to each well.
2. Add 50µL of reconstituted standards or test samples in duplicate to each well.

   Note: If the human IFNγ concentration in any test sample possibly exceeds the highest point on the standard curve, 1000 pg/mL, see Sample Preparation – Sample Dilution Section.
3. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.

4. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate for two (2) hours at room temperature, 20-25°C.
5. Carefully remove adhesive plate cover. Wash plate as described in the Plate Washing section below.

B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.

   Note: For automated washing, aspirate all wells and wash THREE times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution immediately before use. Do not prepare more solution than required. Do not store prepared Streptavidin-HRP Solution.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- Use new reagent reservoir and pipette tips when adding the prepared Streptavidin-HRP Solution.

1. Centrifuge Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5µL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

   For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.
3. Add 100µL of prepared Streptavidin-HRP Solution to each well.
4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
5. Carefully remove plate cover and discard plate contents. Wash plate as described in the Plate Washing section (section B).

D. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate Solution.
- **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate.

1. Pipette 100µL of TMB Substrate into each well.
2. Allow color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
3. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

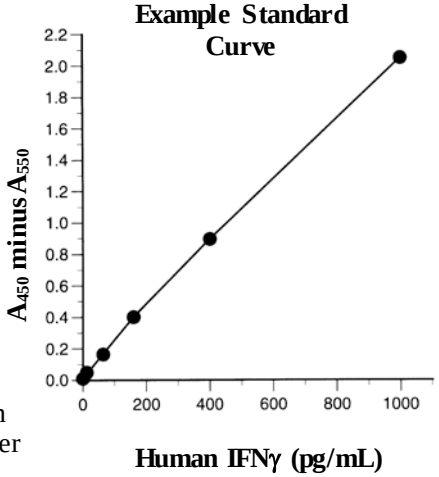
E. Absorbance Measurement

- Evaluate plate within 30 minutes of stopping the reaction.
- Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If 550nm is not available, measure absorbance at 450nm only. Omitting the 550nm measurement results in higher absorbance values.

F. Calculation of Results

- Use the standard curve to determine IFNγ amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding IFNγ concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the human IFNγ amount in each sample by interpolating from the absorbance value (Y axis) to IFNγ concentration (X axis) using the standard curve.
- If the sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to determine amount of IFNγ in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Example Standard Curve

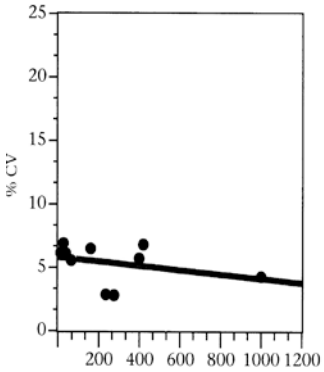


Performance Characteristics

Sensitivity: < 2pg/mL
The sensitivity or Lower Limit of Detection (LLD)[1] was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Assay Range: 25.6-1000 pg/mL
Suggested standard curve points are 1000, 400, 160, 64, 25.6, and 0pg/mL.

Precision: The intra-assay coefficient of variation was plotted against IFNγ concentration (pg/ml). The points represent samples evaluated in replicates of four in four different kit lots.



Reproducibility:
Intra-assay CV: < 10%
Inter-assay CV: < 10%

Specificity: This ELISA is specific for natural and recombinant human IFNγ. It does not cross-react with human IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, TNFα or GM-CSF.

Calibration: The standards in this ELISA have been calibrated to the NIAID recombinant IFNγ standard lot Gxg23-902-535. One (1) pg of Endogen Standard = 0.03 NIAID units.

Recovery: Low and high levels of recombinant human IFNγ were spiked into normal human serum, plasma and urine samples as well as a control buffer. Mean recoveries are as follows:

Spike Level	900pg/mL	150pg/mL
Mean Serum Recovery	106%	84%
Mean Plasma Recovery	95%	65%
Mean Urine Recovery	105%	80%

Expected Values: Serum, plasma and urine samples were collected from apparently healthy individuals and evaluated in this assay. The levels of human IFNγ detected are as follows:

	Mean	Range
Serum (n=35)	< 2pg/mL	< 2pg/mL
Plasma (n=45)	< 2pg/mL	< 2-3pg/mL
Urine (n=5)	< 2pg/mL	< 2pg/mL

Reference

1. *Immunoassay: A Practical Guide*, ed. Chan and Perlstein, 1987, Academic Press. p.71.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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