# AssayMax Human Interferon-γ (IFN-γ) ELISA Kit

## Catalog No. EI1023-1

## Introduction

Interferon-γ (IFN-γ) is a highly pleiotropic protein secreted mainly by activated T-lymphocytes and natural killer cells. It is involved in a wide range of physiological processes, including antiviral, immunoregulatory and anti-tumour properties, cell proliferation and apoptosis, as well as the stimulation and repression of a variety of genes (1 - 3). IFN-γ is a homodimer consisting of two 143-amino-acid polypeptides with 20 kDa and 25 kDa (4). By binding to the receptors IFNGR1 & IFNGR2, IFN-γ activates the tyrosine kinase JAK-STAT pathway (5). While protecting against tumor development and cancer immunoediting, IFN-γ function is significant in tumor surveillance (6). Aside from functions in host defense, IFN-γ may contribute to autoimmune pathology. In humans, IFN-γ is implicated in pathology of diseases such as systemic lupus erythematosus (7), multiple sclerosis (8), and insulin-dependent diabetes mellitus (9). Therapeutically, IFN-γ administration enhances bone resorption and leukocyte function in patients with osteopetrosis (10).

## Principal of the Assay

The AssayMax Human IFN-γ ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human IFN-γ in plasma, serum and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human IFN-γ in less than 4 hours. A polyclonal antibody specific for human IFN-γ has been pre-coated onto a 96-well microplate with removable strips. IFN-γ in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for IFN-γ, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

## Reagents

- **Human IFN-γ Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human IFN-γ.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human IFN-γ Standard:** Human IFN-γ in a buffered protein base (2 ng, lyophilized).
- **Biotinylated IFN-γ Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against IFN-γ (80 μl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

## Storage Condition

- Store kit at 2-8$^0$C or -20$^0$C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8$^0$C. Store reconstituted reagents at -20$^0$C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 μl, 20-200 μl, 200-1000μl and multiple channel)
- Deionized or distilled reagent grade water

## Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. The undiluted samples can be stored at -20$^0$C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. The undiluted samples can be stored at -20$^0$C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20$^0$C or below. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8$^0$C.
- **Standard Curve:** Reconstitute the 2 ng of IFN-γ Standard with 2 ml of EIA Diluent to generate a solution of 1 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting

the standard solution (1 ng/ml) 1:2 with EIA Diluent to produce 0.5, 0.25, 0.125, 0.0625, 0.0313 and 0.016 ng/ml solutions.  EIA Diluent serves as the zero standard (0 ng/ml).  Any remaining solution should be frozen at -20$^0$C.

| Standard Point | Dilution | [IFN-$\gamma$] (ng/ml) |
|---|---|---|
| P1 | Standard (1 ng/ml) | 1.000 |
| P2 | 1 part P1 + 1 part EIA Diluent | 0.500 |
| P3 | 1 part P2 + 1 part EIA Diluent | 0.250 |
| P4 | 1 part P3 + 1 part EIA Diluent | 0.125 |
| P5 | 1 part P4 + 1 part EIA Diluent | 0.063 |
| P6 | 1 part P5 + 1 part EIA Diluent | 0.031 |
| P7 | 1 part P6 + 1 part EIA Diluent | 0.016 |
| P8 | EIA Diluent | 0.000 |

- **Biotin IFN-$\gamma$ Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.  Any remaining solution should be frozen at -20$^0$C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):**  Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.  Any remaining solution should be frozen at -20$^0$C.

## Assay Procedure

- Prepare all reagents, working standards and samples as instructed.  Bring all reagents to room temperature before use.  The assay is performed at room temperature (20-30$^0$C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside.  Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of IFN-$\gamma$ standard or sample per well. Cover wells with a sealing tape and incubate for two hours.  Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated IFN-$\gamma$ Antibody to each well and incubate for two hours.
- Wash a microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops.  Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well.  The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately.  If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some

unstable black particles may be generated at high concentration points after stopping the
reaction for about 10 minutes, which will reduce the readings.

## Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis
  and the corresponding mean 450 nm absorbance on the y-axis.  The best-fit line can be
  determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the
  value by the dilution factor.

## Standard Curve

- The curve is provided for illustration only.  A standard curve should be generated each time
  the assay is performed.

H. IFN-γ Standard Curve
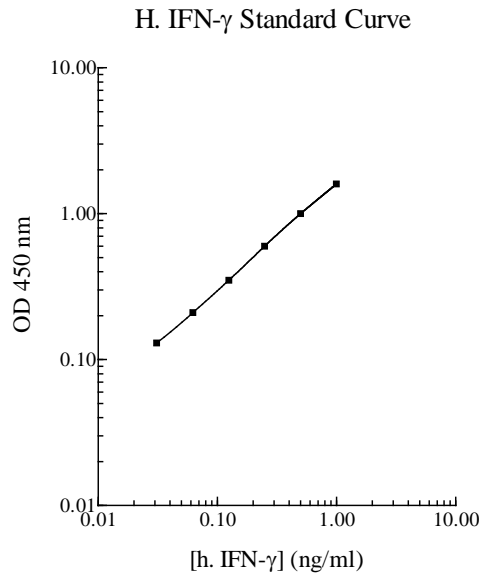


## Performance Characteristics

- The minimum detectable dose of IFN-γ is typically 16 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.4 % and 7.3 % respectively.

## Linearity

| Sample Dilution | Average Percentage of Expected Value | |
| --- | --- | --- |
| | Plasma | Serum |
| No Dilution | 100% | 99% |
| 1:5 | 98% | 103% |
| 1:10 | 101% | 101% |

## Recovery

| | |
|---|---|
| **Standard Added Value** | 0.05 – 0.5 ng/ml |
| **Recovery %** | 89 – 111 % |
| **Average Recovery %** | 98 % |

## Cross-Reactivity

| Species | % Cross Reactivity |
|---|---|
| Beagle | None |
| Bovine | None |
| Monkey | < 10 |
| Mouse | None |
| Rat | None |
| Rabbit | None |

## References

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