**ELISA kits available from ADI:**

| Catalog# | ProdDescription |
|---|---|
| 600-400-CTN | Dog Cardiac Troponin 1 (Tn-I) ELISA Kit |
| 600-410-CTN | Human Cardiac Troponin 1 (Tn-I) ELISA Kit |
| 600-420-CTN | Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit |
| 600-430-MTN | Monkey Skeletal Muscle Troponin1 (Tn-I) ELISA Kit |
| 600-440-CTN | Mouse Cardiac Tn-I ELISA kit for plasma samples |
| 600-450-CTN | Mouse Cardiac Troponin 1 (Tn-I) ELISA Kit |
| 600-470-CTN | Pig Cardiac Troponin 1 (Tn-I) ELISA Kit |
| 600-480-CTN | Rabbit Cardiac Tn-I ELISA kit for serum samples |
| 600-510-MTN | Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit |
| 600-600-DMY | Dog Myoglobin ELISA Kit |
| 600-610-HMY | Human Myoglobin ELISA Kit |
| 600-620-MMY | Monkey Myoglobin ELISA Kit |
| 600-630-MMY | Mouse Myoglobin ELISA Kit |
| 600-640-PMY | Pig Myoglobin ELISA Kit |
| 600-650-RMY | Rabbit Myoglobin ELISA Kit |
| 600-660-RMY | Rat Myoglobin ELISA Kit |

**Human**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

**Monkey**: IgM, IgG, IgA, IgE
**Rat**: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein
**Mouse**: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

**Autoimmune** Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

**Chicken**: IgG, IgM, IgY, Ovalbumin **Turkey**: IgG
**Bovine**: Albumin, IgG, IgM, Lactoferrin, Transferrin
**Pig**: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM
**Cat**: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG

*See Details at the web site or Contact ADI*



*Instruction Manual No. M-100280-IFG*

# Mouse Interferon Gamma (IFG)

## ELISA KIT Cat. # 100-280-IFG

**For Quantitative Determination of Interferon Gamma in Mouse Serum/Plasma**

ALPHA DIAGNOSTIC INTERNATIONAL

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# Mouse Interferon Gamma (IFG) ELISA KIT
## Cat. No. 100-280-IFG

| Kit Components, 96 tests | |
|---|---|
| Anti-Mouse IFG coated strip plate (8 wells x 12 strips) #100280-1 | 1 plates |
| Strip Plate Sealers, #100280-2 | 4 |
| Mouse IFG Reference Standard, 100,000 pg/ml, 55 uL. #100280-3 | 1 vial |
| Anti-mouse IFG Antibody Concentrate #100280-4 | 1 vial |
| Anti-Mouse IFG-HRP Conjugate #100280-5 | I vial |
| HRP Conjugate Diluent, 15 ml, #100280-6 | 1 bottle |
| Dilution Buffer, 50 ml, #100280-7 | 1 bottle |
| Wash Buffer (20x), 50 ml, #100280-WB | 1 bottle |
| TMB Substrate, 15 ml, #100280-TMB | 1 bottle |
| Stop solution, 15 ml, #100280-SS | 1 bottle |
| Instruction Manual, # M-100280 | 1 manual |

## INTRODUCTION

Interferon-gamma (IFG) is a dimerized soluble cytokine that is the only member of the type II class of interferons. This interferon was originally called macrophage-activating factor, a term now used to describe a larger family of proteins to which IFG belongs. IFG has a wide spectrum of biological activities, ranging from the ability to induce an antiviral state in cells to a variety of effects important in regulating responses.

IFG 1b is used to treat chronic granulomatous disease and osteopetrosis. IFG has been shown to interact with Interferon gamma receptor 1.

Mouse IFG is a ~20 kDa factor produced by activated T, B and NK cells, and is an anti-viral and anti-parasitic cytokine. IFG, in synergy with other cytokines such as TNF-α, inhibits proliferation of normal and transformed cells. Immunomodulatory effects of IFG are exerted on a wide range of cell types expressing the high affinity receptors for IFG. Glycosylation of IFG does not affect its biological activity.

Monoclonal antibody to mouse gamma globulin binds both natural and recombinant mouse gamma Interferon. Its binding activity has been demonstrated in vitro and in vivo. These antibodies have been demonstrated to be able to inhibit inflammatory responses to bacterial lipopolysaccharides. These antibodies were furthermore shown to inhibit Shwartzman reactions and to protect NZB mice against spontaneous development of autoimmune disease. The neutralizing activity of the antibody has been demonstrated as being poor in anti-viral assays.

ADI's Mouse IFG ELISA provides is a rapid, specific and sensitive assay for measuring Mouse IFG in serum or other biological fluids.



## CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate IFG concentrations. Read off the IFG concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:20K then the values must be multiplied by 20,000 and results are expressed as ug/ml.

If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to- point fit.

## PERFORMANCE CHARACTERISTICS

**Detection Limit:** The minimum IFG concentration detectable using this assay is below 25 pg/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

**Expected Values**: Each laboratory should establish testing ranges for the animal population being investigated.

**Specificity:** The antibodies used in this kit are specific for Mouse IFG and have shown no cross-reactivity with other IFGs or proteins.

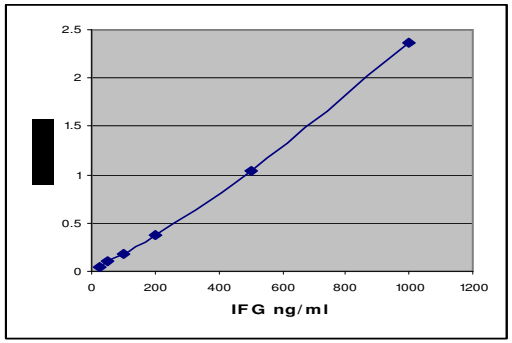
**Species Crossreactivity**: No cross-reactivity of Mouse IFG ELISA kit with human interferon gamma, alpha or beta.

**NOTES:** Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8$^{O}$C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

## WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples | | Mean $A_{450\ nm}$ | Calculated Concn |
|---|---|---|---|---|
| A1, A2 | **Neg. Control** | 0 pg/ml | 0.084 | |
| B1, B2 | **Standard A** | 25 pg/ml | 0.135 | |
| C1, C2 | **Standard B** | 50 pg/ml | 0.187 | |
| D1, D2 | **Standard C** | 100 pg/ml | 0.265 | |
| E1, E2 | **Standard D** | 200 pg/ml | 0.466 | |
| F1, F2 | **Standard E** | 500 pg/ml | 1.130 | |
| G1, G2 | **Standard F** | 1000 pg/ml | 2.455 | |
| H1, H2 | **Sample 1** | | 1.118 | 480 pg/ml |

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.




A typical assay Curve (do not use this for calculating sample values)



## PRINCIPLE OF THE TEST

Mouse IFG ELISA kit is based on binding of Mouse IFG from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of IFG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of IFG in samples and control is read off the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 uL) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

The Mouse IFG ELISA Kit is for research use only.

Standard, antibody and conjugate solutions contain 0.05% thimerosal as a preservative; they should be handled with appropriate safety precautions and discarded properly. Since thimerosal is highly toxic through skin contact, inhalation or ingestion, suitable protective wear and care should be used in handling these solutions.

Stop Solution contains diluted sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for thimerosal, TMB, and sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

## SPECIMEN COLLECTION and HANDLING

Collect blood by venipuncture; allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. Plasma can also be used. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. Cell or tissues extract samples have not been optimized.

## REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit.

2. Standards, antibody and conjugate should be diluted as shown on page 3.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8$^{o}$C until the expiration date printed on the label.

## DILUTION OF SAMPLES

Samples with IFG values greater than 1000 pg/ml should be diluted 5-fold prior to assay. For example, use 50 uL of serum or plasma and dilute it with 200 uL of sample diluent (1X).

## TEST PROCEDURE

*(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. Then concentration of the reference standard is 100,000 pg/ml. Aliquot and stores any unused reference standard at –20oC or below.

2. Prepare liquid standards using the following dilution scheme. Label 7 microcentrifuge tubes as 1000, 500, 200, 100, 50, 25 and 0 pg/ml.

3. For standard F (1000 pg/ml) pipette the volume of IFG diluent and add the indicated volume of IFG reference standard and mix gently. Prepare the remaining standards as shown below.

| Mouse IFG Stds | Stock Volume | IFG diluent | Final Volume |
|---|---|---|---|
| **Std F** (1000 pg/ml) | 10 uL of Ref. | 990 uL | 1000 uL |
| **Std E** (500 pg/ml) | 500 uL of Std F | 500 uL | 1000 uL |
| **Std D** (200 pg/ml) | 600 uL of Std E | 400 uL | 1000 uL |
| **Std C** (100 pg/ml) | 500 uL of Std D | 500 uL | 1000 uL |
| **Std B** (50 pg/ml) | 500 uL of Std C | 500 uL | 1000 uL |
| **Std A** (25 pg/ml) | 500 uL of Std B | 500 uL | 1000 uL |
| **Negative** (0 pg/ml) | 0 | 500 uL | 500 uL |

**Notes**: When preparing the serial dilutions of the standards, gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme, you will have 500 uL of negative and standards (B-F) except for 400uL for standard E and 1000 uL of Std. A. You would need 200 uL of each standard (100 uL in duplicate).



Label or mark the microtiter well strips to be used on the plate.

4. Pipet **100 uL standards and diluted samples** into appropriate wells. Cover the plate with the plate sealer. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes in a closed chamber,** such as a drawer.

5. Remove or aspirate the plate contents and **wash the wells one time only** with 300 uL of 1x wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds. Tap the plate over fresh paper towels after washing.

6. For each strip dilute 5 uL of the antibody concentrate with 1.0 ml of dilution buffer. Pipette **100 uL of diluted antibody solution** into each well. Mix gently, and incubate at room temperature (20-25oC) for 6**0 minutes in an enclosed chamber.**

7. **Repeat step 5 above for washing x 3.**

8. For each strip dilute 4 uL of the Anti-Mouse IFG-HRP Conjugate with 1.0 ml of conjugate diluent. Aliquot and store any unused conjugate at –70oC. Pipette **100 uL of the diluted conjugate** into each well. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes in an enclosed chamber.**

9. **Repeat step 5 above for washing x 4.**

10. Add **100 uL of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **15 minutes in an enclosed chamber in the dark.** Blue color develops. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

11. Stop the reaction by adding **100 uL of stop solution** to all wells. Mix gently. Blue color turns yellow.

12. Measure the **absorbance at 450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

13. Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero troponin-I value