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

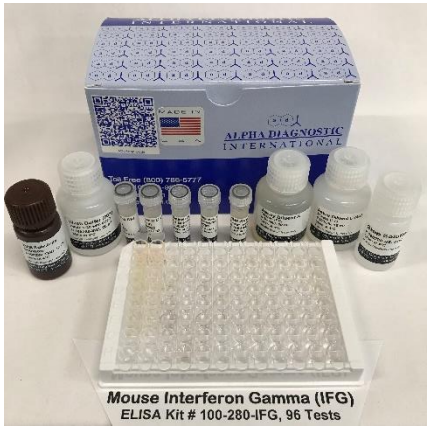
See Details at the web site or Contact ADI

Instruction Manual No. M-100-280-IFG

Mouse Interferon Gamma (IFG)

ELISA KIT Cat. # 100-280-IFG, 96 Tests

For Quantitative Determination of Interferon Gamma in Mouse Serum/Plasma



For In-vitro research use only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: Techsupport@4adi.com

Web Site: www.4adi.com

Mouse Interferon Gamma (IFG) ELISA KIT
Cat. No. 100-280-IFG, 96 Tests

Kit Components, 96 tests	
Anti-Mouse IFG **coated strip plate** (8 wells x 12 strips) #100281 **Recommended storage -20°C**	1 plate
Mouse IFG Standard protein, 1 vial is enough to run each standard in duplicate #100282	2 vials
Biotinylated Anti-mouse IFG Antibody. Each vial is enough to assay half the microplate #100283	2 vials
HRP-Streptavidin concentrate, 0.2 ml (440x) #100284	I vial
Assay Diluent A, 30 ml, #100285	1 bottle
Assay Diluent B, 15 ml (5X), #100286	1 bottle
Wash Buffer (20x), 25 ml, #100280-WB	1 bottle
TMB Substrate Solution, 12 ml, #100280-TMB	1 bottle
Stop solution, 8 ml, #100280-SS	1 bottle
Instruction Manual, # M-100280	1 manual

Intended Use:

ADI's Mouse IFG ELISA provides a rapid, specific and sensitive assay for measuring Mouse IFG in serum or other biological fluids. **For In-vitro research use only(RUO)**.

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.

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.

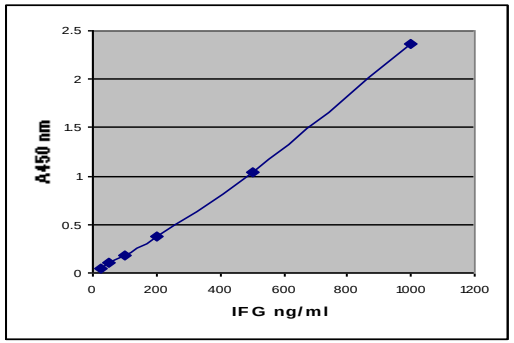
Recommended Dilution (serum & plasma): 2 fold

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 assay employs an antibody specific for mouse IFN-gamma coated on a 96 well plate. Standards and samples are pipetted into the wells and IFN-gamma present in the sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IFN-gamma antibody is added. After washing, HRP-conjugated streptavidin is pipetted to the wells. 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

Bring all reagents and samples to room temperature before use.

1. **Dilute Wash Buffer (20x stock)**. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
2. **Assay Diluent B** should be diluted 5-fold with deionized or distilled water before use.
3. **Sample dilution**: **Assay Diluent A** should be used for dilution of serum and plasma samples. 1X Assay Diluent B should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold. Note: Levels of IFN-gamma may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Briefly spin the **Detection Antibody vial (#100280-3)** 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 4ºC for 5 days). The detection antibody concentrate should be diluted 120-fold with 1X Assay Diluent B and used in step 6 of test Procedure.
5. Briefly spin the **HRP-Streptavidin concentrate vial (#100280-4)** and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 440-fold with 1X Assay Diluent B.

For example: Add 25 µl of HRP-Streptavidin concentrate into a tube with 11 ml 1X Assay Diluent B to prepare a 440-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

STORAGE AND STABILITY

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.

TEST PROCEDURE

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

1. Briefly spin a vial of Mouse IFG Standard protein #100280-2. Add 400µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into #100280-2 vial to prepare a 20 ng/ml standard. Dissolve the powder thoroughly by a gentle mix.

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 diluent and add the indicated volume of IFG standard protein (#100280-2) and mix gently. Prepare the remaining standards as shown below.

Mouse IFG Stds	Stock Volume	Assay diluent	Final Volume
Std F (1000 pg/ml)	50 uL of std. (#100280-2)	950 uL	1000 uL
Std E (500 pg/ml)	500 uL of Std F	500 uL	1000 uL
Std D (200 pg/ml)	400 uL of Std E	600 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 for **2.5 hours** at room temperature (20-25°C) with gentle shaking.

5. Remove or aspirate the plate contents and **wash the wells four times** 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. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 4) to each well. Incubate for 1 hour at room temperature with gentle shaking.

7. **Discard the solution. Repeat step 5 above for washing.**

8. Add 100 µl of prepared Streptavidin HRP solution (see Reagent Preparation step 5) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

9. **Discard the solution. Repeat step 5 above for washing.**

10. Add **100 uL of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **30 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 **50 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