Ifng (Mouse) ELISA Kit

Catalog Number KA0242

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The Ifng (Mouse) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of mouse IFN-γ. The mouse IFN-γ ELISA is for research use only. Not for diagnostic or therapeutic procedures.

Background

IFN-γ, also called Type II interferon, is a homodimeric gycoprotein containing approximately 21 to 24 kD subunits.

In contrast to IFNα and IFNβ synthesis, which can occur in any cell, production of IFN-γis a function of T cells and NK cells. All IFN-γ inducers activate T cells either in a polyclonal (mitogens or antibodies) or in a clonally restricted, antigen-specific, manner. IFN-γ is produced during infection by T cells of the cytotxic/suppressor phenotype (CD8) and by a subtype of helper T cells, the Th1 cells. Th1 cells secrete IL-2, IL-3, TNFβ and IFN-γ, whereas Th2 cells mainly produce IL-3, IL-4, IL-5 and IL-10, but little or no IFN-γ. IFN-γ preferentially inhibits the proliferation of Th2 but not Th1 cells, indicating that the presence of I IFN-γ during an immune response will result in the preferential proliferation of Th1 cells.

Type II IFN or IFN-γ is a lymphokine that displays no molecular homology with type I IFN, but shares some important biologic activities. Specifically, IFN-γ induces an anti-viral state and is anti-proliferative. In addition, IFN-γ has several properties related to immunoregulation.

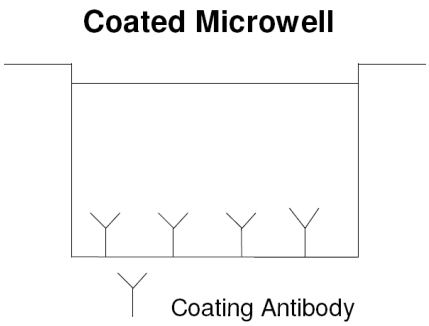
- ✓ IFN-γ is a potent activator of mononuclear phagocytes, e.g. IFN-γ stimulates the expression of Mac-1, augments endocytosis and phagocytosis by monocytes; and activates macrophages to kill tumor cells by releasing reactive oxygen intermediates and TNFα.
- ✓ IFN-γ induces or augments the expression of MHC antigens on macrophages, T and B cells and some tumor cell lines.
- ✓ On T and B cells IFN-γ promotes differentiation. It enhaces proliferation of activated B cells and can act synergistically with IL-2 to increase immunoglobulin light-chain synthesis. IFN-γ is one of the natural B-cell differentiation factors.
- ✓ Finally, IFN-γ activates neutrophils, NK cells and vascular endothelial cells.

The role of IFN-γ as a disease marker has been demonstrated for a number of different pathological situations.

Principle of the Assay

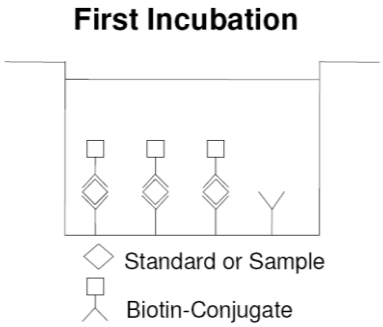
An anti-mouse IFN-γ coating antibody is adsorbed onto microwells.

Figure 1



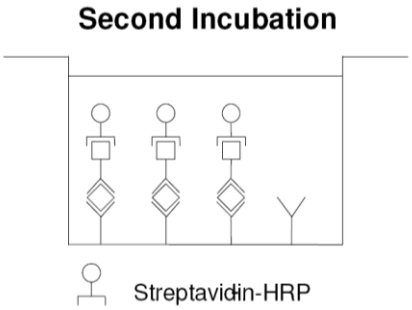
Mouse IFN-γ present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-mouse IFN-γ antibody is added and binds to mouse IFN-γ captured by the first antibody.

Figure 2



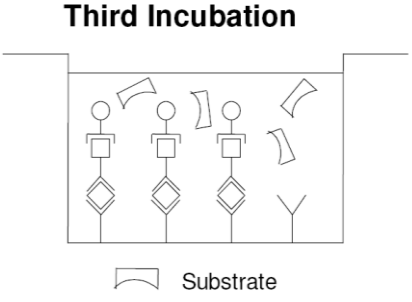
Following incubation unbound biotin-conjugated anti-mouse IFN-γ antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-mouse IFN-γ antibody.

Figure 3



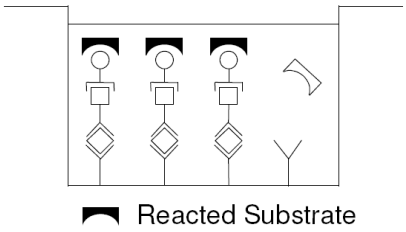
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4



A coloured product is formed in proportion to the amount of mouse IFN-γ present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 mouse IFN-γ standard dilutions and mouse IFN-γ sample concentration determined.

Figure 5



General Information

Materials Supplied

List of component

Component	Amount
Microwell Plate coated with monoclonal antibody to mouse IFN-γ	96(8x12) wells
Biotin-Conjugate anti- mouse IFN-γ monoclonal antibody	100 µL
Streptavidin-HRP	150 µL
Mouse IFN-γ Standard lyophilized, 2 ng/mL upon reconstitution	2 vials
Sample Diluent *Please note: In some cases the Sample Diluent contains an insoluble precipitate which does not interfere in any way with the test performance. Use according to protocol.*	12 mL
Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)	5 mL
Wash Buffer Concentrate 20x (PBS with 1% Tween 20)	50 mL
Substrate Solution (tetramethyl-benzidine)	15 mL
Stop Solution (1M Phosphoric acid)	15 mL
Blue-Dye	0.4 mL
Green-Dye	0.4 mL
Red-Dye	0.4 mL
Adhesive Film	4 slices

Storage Instruction

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Materials Required but Not Supplied

- ✓ 5 mL and 10 mL graduated pipettes
- ✓ 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- ✓ 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- ✓ Multichannel micropipette reservoir
- ✓ Beakers, flasks, cylinders necessary for preparation of reagents
- ✓ Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- ✓ Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- ✓ Glass-distilled or deionized water
- ✓ Statistical calculator with program to perform regression analysis

Precautions for Use

- ✓ All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- ✓ Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- ✓ Do not mix or substitute reagents with those from other lots or other sources.
- ✓ Do not use kit reagents beyond expiration date on label.
- ✓ Do not expose kit reagents to strong light during storage or incubation.
- ✓ Do not pipette by mouth.
- ✓ Do not eat or smoke in areas where kit reagents or samples are handled.
- ✓ Avoid contact of skin or mucous membranes with kit reagents or specimens.
- ✓ Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- ✓ Avoid contact of substrate solution with oxidizing agents and metal.
- ✓ Avoid splashing or generation of aerosols.
- ✓ In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- ✓ Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- ✓ Exposure to acid inactivates the conjugate.
- ✓ Glass-distilled water or deionized water must be used for reagent preparation.
- ✓ Substrate solution must be at room temperature prior to use.
- ✓ Decontaminate and dispose specimens and all potentially contaminated materials as they could contain

infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- ✓ Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

- Limitation

- ✓ Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- ✓ Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- ✓ Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- ✓ Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Assay Protocol

Reagent Preparation

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

- Wash Buffer (1x)

  Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.

  Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days. Wash Buffer(1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

- Assay Buffer (1x)

  Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.

  Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

  Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

- Biotin-Conjugate

  Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

  Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

- Streptavidin-HRP

  Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

  Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

- Mouse IFN-γ Standard

  Reconstitute mouse IFN-γ standard by addition of distilled water.

  Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 2 ng/mL). Allow the reconstituted standard to sit for 10-30 minutes. Mix well prior to making dilutions.

  After usage remaining standard cannot be stored and has to be discarded.

  Standard dilutions can be prepared directly on the microwell plate or alternatively in tubes.

✓ External Standard Dilution

Label 7 tubes, one for each standard point.

S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 µL of Sample Diluent into each tube.

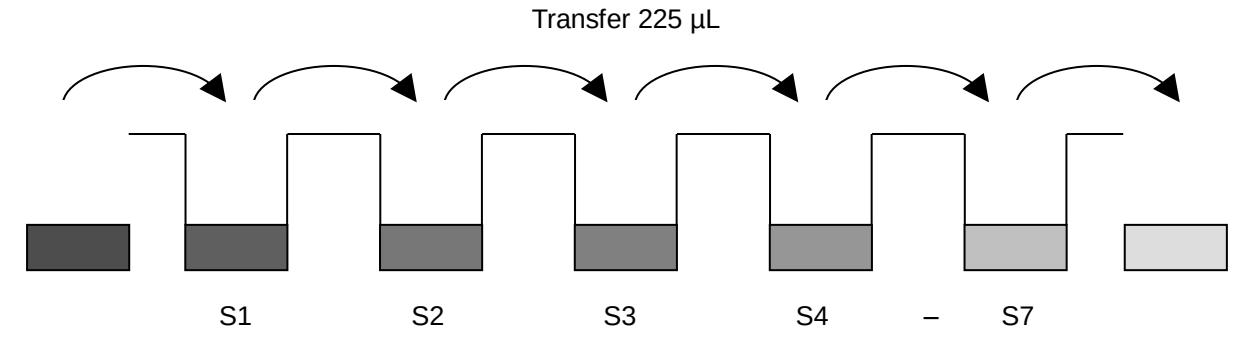
Pipette 225 µL of reconstituted standard (concentration = 2 ng/mL) into the first tube, labelled S1, and mix (concentration of standard 1 = 1 ng/mL).

Pipette 225 µL of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.

Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

Sample Diluent serves as blank.

Figure 6



- Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

  In order to help our customers to avoid any mistakes in pipetting the Abnova ELISAs, Abnova offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

  This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

  Alternatively, the dye solutions from the stocks provided (Blue-Dye, Green-Dye, Red-Dye) can be added to the reagents according to the following guidelines:

✓ Diluent: Before standard and sample dilution add the Blue-Dye at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of Blue-Dye, proceed according to the instruction booklet.

5 mL Sample Diluent	20 µL Blue-Dye
12 mL Sample Diluent	48 µL Blue-Dye
50 mL Sample Diluent	200 µL Blue-Dye

✓ Biotin-Conjugate: Before dilution of the concentrated Biotin-Conjugate, add the Green-Dye at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of Green-Dye according to the instruction booklet: Preparation of Biotin-Conjugate.

3 mL Assay Buffer (1x)	30 µL Green-Dye
6 mL Assay Buffer (1x)	60 µL Green-Dye

✓ Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP, add the Red-Dye at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of Red-Dye according to the instruction booklet: Preparation of Streptavidin-HRP.

6 mL Assay Buffer (1x)	24 µL Red-Dye
12 mL Assay Buffer (1x)	48 µL Red-Dye

Sample Preparation

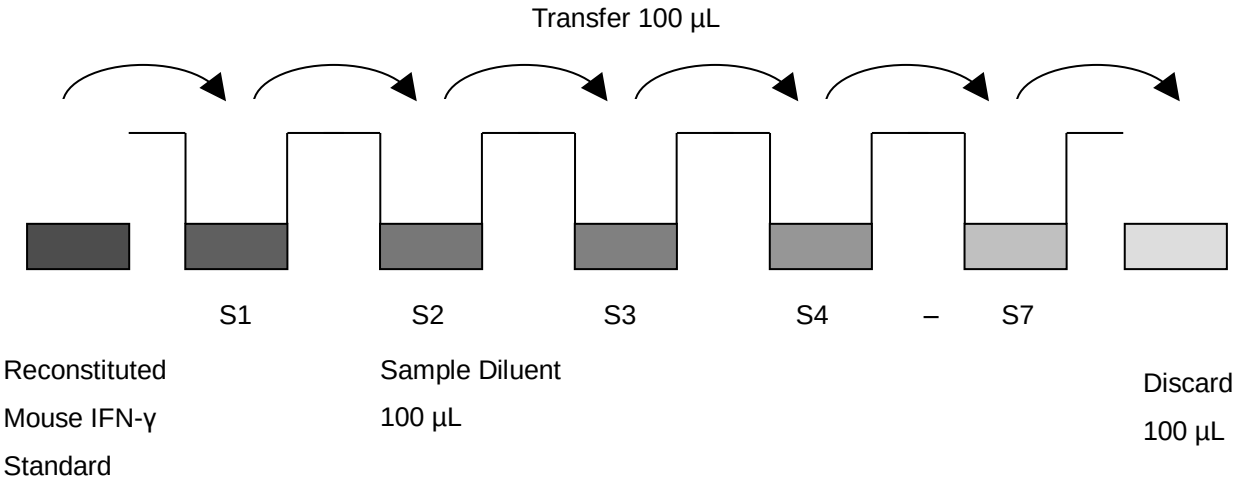
Cell culture supernatant and serum were tested with this assay. Other body fluids might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive mouse IFN-γ. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
2. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microwells.
   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
3. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes. Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see Preparation of Standard, concentration = 2000.0 pg/mL), in duplicate, into well A1 and A2 (see Plate Layout). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1 S1 = 1000.0 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 6 times, creating two rows of mouse IFN-γ standard dilutions, ranging from 1000.0 to 15.6 pg/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 7



In case of an external standard dilution, pipette 100 µL of these standard dilutions (S1 - S7) in the standard wells according to Plate Layout.

4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 50 µL of Sample Diluent to the sample wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see Preparation of Biotin-Conjugate).
8. Add 50 µL of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.
10. Prepare Streptavidin-HRP (refer to Preparation of Streptavidin-HRP).
11. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 2. of the Assay Procedure. Proceed immediately to the next step.
12. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
13. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 400 rpm.
14. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 2. of the Assay Procedure. Proceed immediately to the next step.
15. Pipette 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

    The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable.

    Determination of the ideal time period for colour development has to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 - 0.95.
17. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
18. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

    Note:In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Data Analysis

Calculation of Results

- ✓ Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- ✓ Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the mouse IFN-γ concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- ✓ To determine the concentration of circulating mouse IFN-γ for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding mouse IFN-γ concentration.
- ✓ If instructions in this protocol have been followed samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- ✓ Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low mouse IFN-γ levels (Hook Effect). Such samples require further external predilution according to expected mouse IFN-γ values with Sample Diluent in order to precisely quantitate the actual mouse IFN-γ level.
- ✓ It is suggested that each testing facility establishes a control sample of known mouse IFN-γ concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- ✓ A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 8

Representative standard curve. Mouse IFN-γ was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

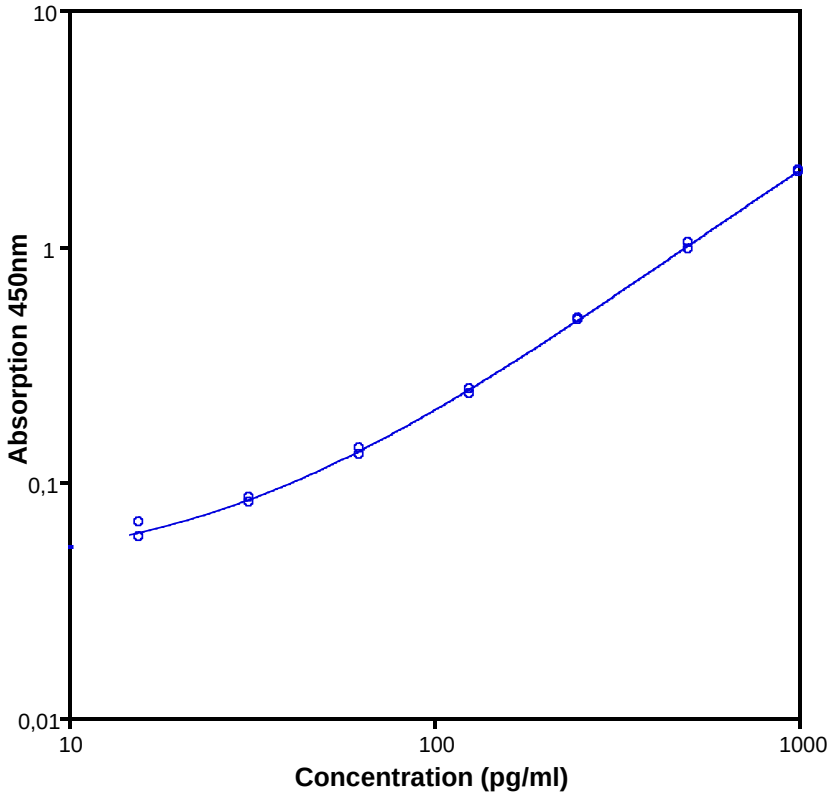


Table 1

Typical data using the Ifng (Mouse) ELISA Kit

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Mouse IFN-γ Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1000.0	2.076	2.066	0.7
	1000.0	2.055		
2	500.0	1.027	0.998	4.2
	500.0	0.968		
3	250.0	0.487	0.490	0.9
	250.0	0.493		
4	125.0	0.246	0.241	2.9
	125.0	0.236		
5	62.5	0.129	0.134	4.8
	62.5	0.138		
6	31.3	0.082	0.084	2.5
	31.3	0.085		
7	15.6	0.067	0.063	10.2
	15.6	0.058		
Blank		0.035	0.031	
		0.026		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

Performance Characteristics

- Sensitivity

  The limit of detection of mouse IFN-γ defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 5.3 pg/mL (mean of 6 independent assays).

- Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse IFN-γ. 2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was < 5%.

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse IFNγ. 2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was < 10%.

- Spike Recovery

  The spike recovery was evaluated by spiking 4 levels of mouse IFN-γ into pooled murine serum. Recoveries were determined in 2 independent experiments with 4 replicates each. The unspiked serum was used as blank in these experiments. The overall mean recovery was 76%.

- Dilution Parallelism

  4 serum samples with different levels of mouse IFN-γ were analysed at serial 2 fold dilutions with 4 replicates each. The overall mean recovery was 107%.

- Sample Stability

a. Freeze-Thaw Stability

Aliquots of spiked serum samples were stored at -20˚C and thawed 5 times, and the mouse IFN-γ levels determined. There was no significant loss of mouse IFN-γ immunoreactivity detected by freezing and thawing.

b. Storage Stability

Aliquots of spiked serum samples were stored at -20˚C, 2-8˚C, room temperature (RT) and at 37˚C, and the mouse IFN-γ level determined after 24 h. There was no significant loss of mouse IFN-γ immunoreactivity detected during storage under above conditions

- Specificity

  The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevant concentrations into a mouse IFN-γ positive serum. There was no crossreactivity detected.

- Expected Values

  There were no detectable mouse IFN-γ levels found.

  Elevated mouse IFN-γ levels depend on the type of immunological disorder.

Resources

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1 (1000.0 pg/mL)	Standard 1 (1000.0 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	Standard 2 (500.0 pg/mL)	Standard 2 (500.0 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	Standard 3 (250.0 pg/mL)	Standard 3 (250.0 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	Standard 4 (125.0 pg/mL)	Standard 4 (125.0 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	Standard 5 (62.5 pg/mL)	Standard 5 (62.5 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	Standard 6 (31.3 pg/mL)	Standard 6 (31.3 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	Standard 7 (15.6 pg/mL)	Standard 7 (15.6 pg/mL)	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	Blank	Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample