



Canine IFN- γ Immunoassay

Catalog Number: EA800007

For the quantitative determination of Canine IFN- γ concentrations in cell culture supernates, serum, and plasma.

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

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TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	1
PRINCIPLE OF THE ASSAY.....	1
TECHNICAL HINTS AND LIMITATIONS.....	2
PRECAUTIONS.....	2
KIT COMPONENTS& STORAGE CONDITIONS.....	3
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	4
SPECIMEN COLLECTION & STORAGE.....	4
REAGENTS PREPARATION.....	4
ASSAY PROCEDURE	6
CALCULATION OF RESULTS.....	6
PERFORMANCE CHARACTERISTICS.....	8
REFERENCES.....	10

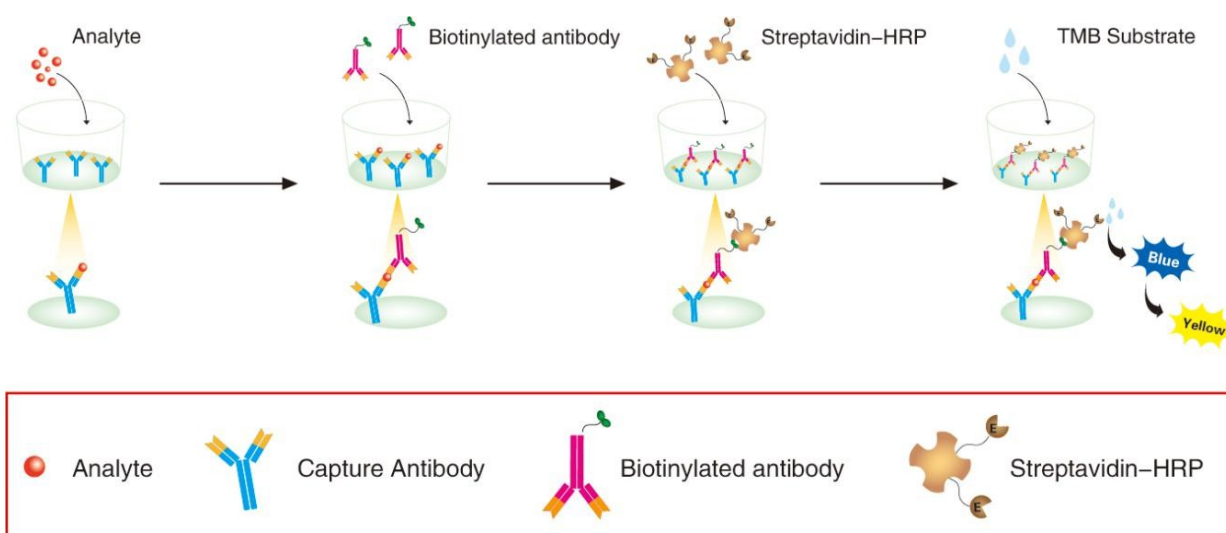
BACKGROUND

IFN- γ is a dimerized soluble cytokine that is the only member of the type II class of interferons . It plays key roles in both the innate and adaptive immune response against viral and intracellular bacterial infections and for tumor control . It alters transcription in up to 30 genes producing a variety of physiological and cellular responses. IFN- γ activates the cytotoxic activity of innate immune cells such as macrophages and NK cells . IFN- γ production by NK cells and antigen-presenting cells (APCs) promotes the cell mediated adaptive immunity by inducing IFN- γ production by T lymphocytes, increased class I and class II MHC expression, and enhancing peptide antigen presentation . The anti-viral activity of IFN- γ is due to its induction of PKR and other regulatory proteins. Binding of IFN- γ to the IFNGR1/IFNGR2 complex promotes dimerization of the receptor complexes to form (IFNGR1/IFNGR2)₂-IFN- γ dimer. Binding induces a conformational change in receptor intracellular domains and signaling involves Jak1, Jak2 and Stat1 . The critical role of IFN- γ in amplification of immune surveillance and function is supported by increased susceptibility to pathogen infection by IFN- γ or IFNGR knockout mice and in humans with inactivating mutations in IFNGR1 or IFNGR2. IFN- γ also appears to have a role in atherosclerosis .

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN- γ present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IFN- γ is added to detect the captured IFN- γ protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:





TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized, 1200pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	2 vials	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C** for six months
Substrate Solution	1 bottle	Store at 2-8°C** for six months
Stop Solution	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.



OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room

temperature or overnight at 2-8°C. Centrifuge approximately for 15 minutes at 1000×g. Assay

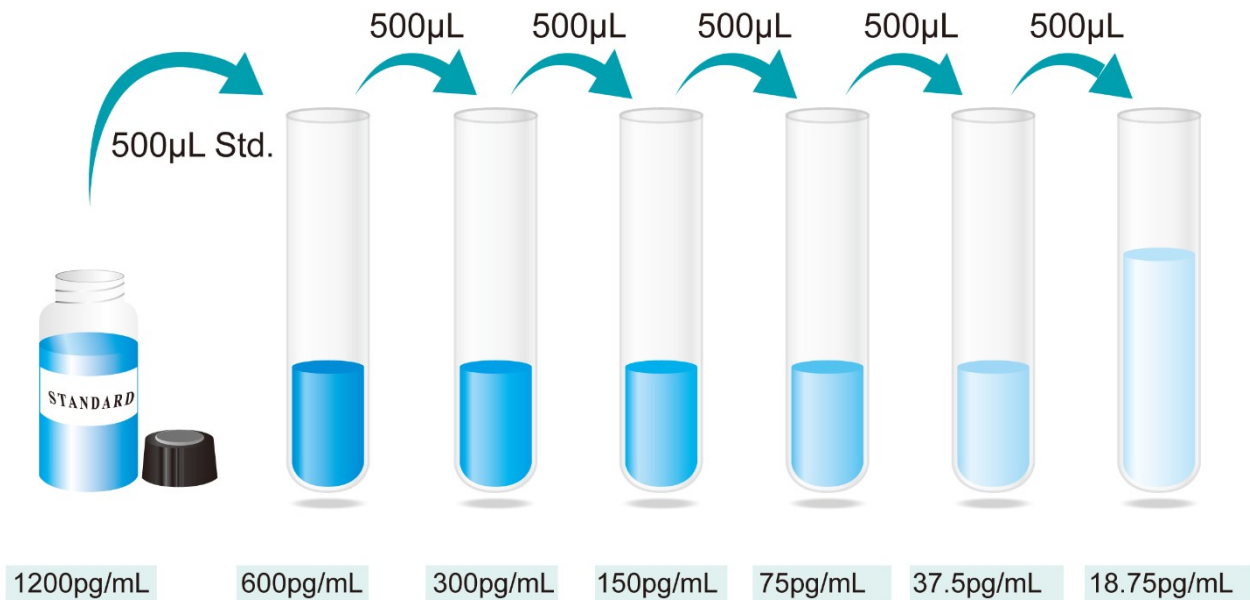
immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal Bovine serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample(2 vials)** - Bovine IFN- γ Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of deionized or distilled water. This reconstitution produces a stock solution of 1200 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 μ L of Standard/Sample Diluent into 600pg/ml tube and the remaining tubes. Use the stock solution of 1200pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly (vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 1200 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Canine IFN- γ standard dilutions

***If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

- Working solution of Biotin-Conjugate anti-Canine IFN- γ antibody(2 vials)** - The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 μ L of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50 μ L of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

- Working solution of Streptavidin-HRP(120 μ L)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 μ L HRP Conjugate sufficient for a 96-well plate. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 μ L of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.



Add 100 μ l standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25 \pm 2 $^{\circ}$ C). Aspirate and wash 4 times



Add 100 μ l working solution of Biotin-Conjugate anti-Canine IFN- γ antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25 \pm 2 $^{\circ}$ C).



Aspirate and wash 4 times

Add 100 μ l working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25 \pm 2 $^{\circ}$ C).



Aspirate and wash 5 times

Add 100 μ l Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25 \pm 2 $^{\circ}$ C).Protect from light.



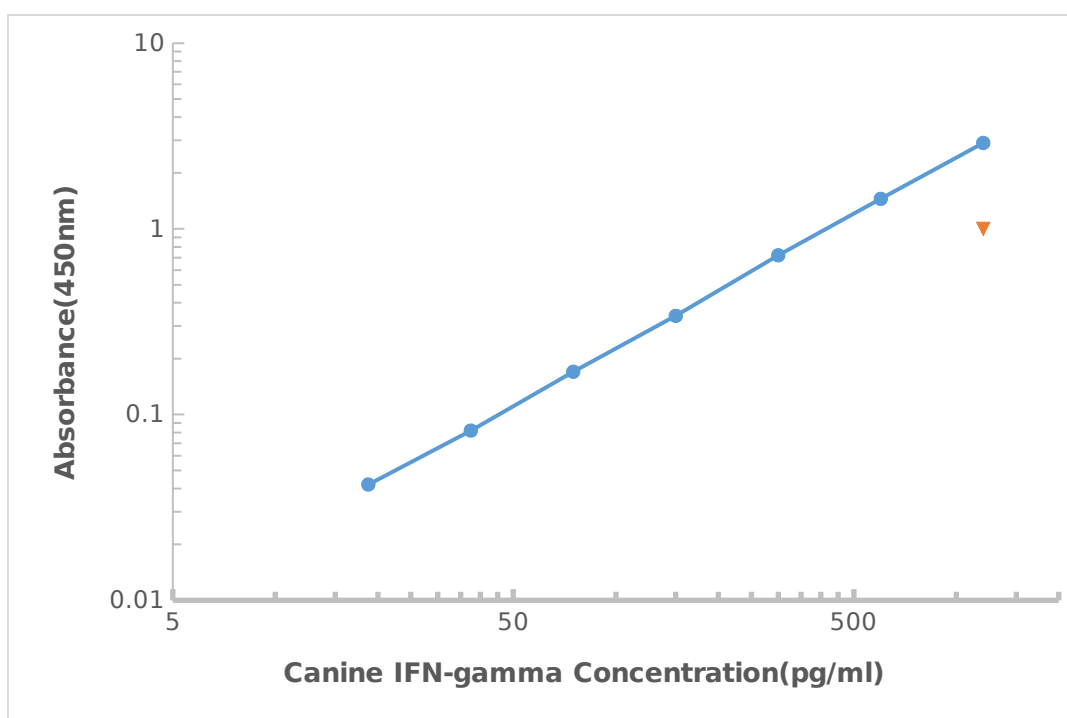
Add 50 μ l Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of samples.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IFN- γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IFN- γ ELISA

Std (pg/mL)	O.D.1	O.D.2	Average	Corrected
0	0.076	0.082	0.079	---
18.75	0.146	0.148	0.147	0.068
37.5	0.216	0.224	0.22	0.141
75	0.345	0.361	0.353	0.274
150	0.526	0.506	0.516	0.437
300	0.936	0.951	0.943	0.864
600	1.587	1.559	1.573	1.494
1200	2.169	2.183	2.176	2.097



Representative standard curve for IFN-γ ELISA

Performance Characteristics



SENSITIVITY: The minimum detectable dose was 10 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Canine IFN- γ . The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant Canine	Recombinant mouse	Recombinant porcine
IL-2	TNF- α	
IL-4	IFN- γ IL-2	
IL-6	IL-4	
IL-1 β	TNF- α	
TNF- α	IFN- γ	

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of IFN- γ spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IFN- γ in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	94	86-102
Cell culture supernatants	95	84-104

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IFN- γ in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were



initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	97	104
	Range (%)	88-108	96-113
1:4	Average% of Expected	95	106
	Range (%)	87-103	94-116

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