Canine IFN gamma ELISA kit

Canine IFN gamma ELISA kit is an Enzyme Immunoassay kit for the quantification of Canine IFN gamma in serum, plasma and cell culture supernatants.

Catalog number: ARG82959

Package: 96 wells

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

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INTRODUCTION

Interferon gamma (IFN-γ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. The existence of this interferon, which early in its history was known as immune interferon, was described by E. F. Wheelock as a product of human leukocytes stimulated with phytohemagglutinin, and by others as a product of antigen-stimulated lymphocytes. It was also shown to be produced in human lymphocytes or tuberculin-sensitized mouse peritoneal lymphocytes challenged with Mantoux test (PPD); the resulting supernatants were shown to inhibit growth of vesicular stomatitis virus. Those reports also contained the basic observation underlying the now widely employed IFN-γ release assay used to test for tuberculosis. In humans, the IFN-γ protein is encoded by the IFNG gene. [Provide by Wikipedia: Interferon gamma]

PRINCIPLE OF THE ASSAY

This Canine IFN gamma ELISA kit is a quantitative sandwich enzyme immunoassay that measures the amount of Canine IFN gamma in the samples. An antibody specific for Canine IFN gamma has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN gamma present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Canine IFN gamma is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of IFN gamma

bound in the initial step. The color development is stopped, and the intensity of the color is measured.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
20X Wash Buffer	50 mL	4°C
Standard (lyophilized)	3 vial (1 ng each vial)	-20°C
Standard & Sample Diluent Buffer	20 mL	4°C
30X Antibody Conjugate	1 vial	4°C
Antibody Conjugate Diluent Buffer	16 mL	4°C
30X HRP Conjugate	1 vial	4°C
HRP Conjugate Diluent Buffer	16 mL	4°C
TMB Substrate	12 mL	4°C (protect from light)
Stop Solution	12 mL	4°C
Plate sealer	6 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Mixer or Ultra-Turrax
- 37°C incubator
- Pipettes and pipette tips
- Microtiter plate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (22-25°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 40°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 1,000 x g for 15 minutes at 4°C.

Plasma: Collect blood with EDTA and centrifuge at 1,000 x g for 15 minutes at 4°C.

Cell culture supernatant: Centrifuge at 300 x g for 10 minutes at 4°C to remove the cell debris.

Note:

- Samples containing sodium azide should not be used in the assay.
- Do not use haemolytic, icteric or lipaemic specimens.
- Specimens should be capped and may be stored for up to one week at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen at <-20°C prior to assay. Thawed samples should be inverted several times prior to testing.
- If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Diluent Buffer and re-assayed.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 2 weeks at room temperature.
- **1X Antibody Conjugate:** Dilute 30X Antibody conjugate into Antibody Conjugate Diluent Buffer to yield 1X Antibody Conjugate. Preparation in 20 minutes before the assay.
- **1X HRP Conjugate:** Dilute 30X HRP conjugate into HRP Conjugate Diluent Buffer to yield 1X HRP Conjugate. Preparation in 20 minutes before the assay.
- **Standard:** add 1 mL of Standard & Sample Diluent Buffer to the standard vial. Allow all component to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution.

Standard tube	IFN gamma (pg/mL)	Standard & Sample Diluent Buffer (μL)	Standard stock, 1000 pg/mL (μL)
S1	1000	0	1000
S2	500	500	500 of S1
S3	250	500	500 of S2
S4	125	500	500 of S3
S5	62.5	500	500 of S4
S6	31.3	500	500 of S5
S7	15.6	500	500 of S6
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **Standards and prepared samples** into the appropriate wells of the Antibody Coated Microplate.
2. Incubate at **37ºC** for **60 minutes.**
3. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (350 µL)** using a squirt bottle, manifold dispenser, or autowasher. Keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the **1X Antibody Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate at **37ºC** for **30 minutes.**
5. Aspirate and wash plate as in step 3.
6. Add **100 µL** of the **1X HRP Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate at **37ºC** for **20 minutes.**
7. Aspirate and wash plate as in step 3.
8. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **15 minutes** at **37ºC**.
9. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 3 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Subtract Blank value from all standards and samples to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

The sensitivity of the Canine IFN gamma ELISA kit is 7.8 pg/mL.

Specificity

This kit do not cross-reacting with Canine IFN-γR1, IFN-α, IFN-β; Human IFN-γ, IFN-γ R1, IFN-α, IFN-β; Mouse IFN-γ, IFN-γ R1, IFN-α; Goat IFN-γ, IFN-γ R1.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was ≤ 5.8% and CV value of inter-assay precision was ≤ 6.9%.