Chicken IFN-γ ELISA Kit

IFN-γ is a dimerized soluble cytokine that is the only member of the type II class of interferons (1). It plays key roles in both the innate and adaptive immune response against viral and intracellular bacterial infections and for tumor control (2). 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 (3,4). 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 (3). 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 (5). 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 (6).

References

1. Gray PW and Goeddel DV (1982). Nature 298 (5877): 859–63.
2. Schroder K, et al (2004). J. Leukoc. Biol. 75 (2): 163–89.
3. Schroder, K. et al. (2004) J Leukoc Biol 75, 163-89.
4. Martinez, F.O. et al. (2009) Annu Rev Immunol 27, 451-83.
5. Kotenko, S.V. et al. (1995) J Biol Chem 270, 20915-21.
6. McLaren, J.E. and Ramji, D.P. (2009) Cytokine Growth Factor Rev 20, 125-35.

PRINCIPLE OF THE ASSAY

This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for Chicken IFNγ has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFNγ present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Chicken IFNγ 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 IFNγ bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

Storage

Store the standard and detection antibody at 4°C ~ -20°C, 10 x reagent diluents and the antibody coated plate at -20°C and store the rest of the kit at 4°C. The kit can be used in 3 months.

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	10 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	Datasheet/Manual	1
Standard	3			96-well plate sheet	1

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with Chicken IFNγ capture antibody-**Store at -20°C upon receipt.

Chicken IFNγ Detection Antibody– 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. The vial contains sufficient Detection Antibody for a 96-well plate. Centrifuge 1 min at 6000 x g prior to open the vial. Add 200 µL of 1 x Reagent Diluent, vortex 20 sec and allow it to sit for 5 min prior to use. Take the entire 200 µL of detection antibody to 9.8 mL Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Chicken IFNγ Standard (3 vials) – The lyophilized Chicken IFNγ Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C ~ -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 µL of 1 x Reagent Diluent to a Standard vial to make the high standard concentration of 1,000 pg /ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Reagent Diluent, vortex 20 sec for each of dilution steps.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Make 1:200 dilution in Reagent Diluent. If the entire 96-well plate is used, add all 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 2 - 8° C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Wash Buffer, 25 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – Store at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 µL of sample or standard to each well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each dilution step is crucial to ensure a normal standard curve.
2. If IFNγ exceeds the upper limit of the detection, the sample needs to be diluted with PBS. Dilution factor must be used for calculation of the analyte concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

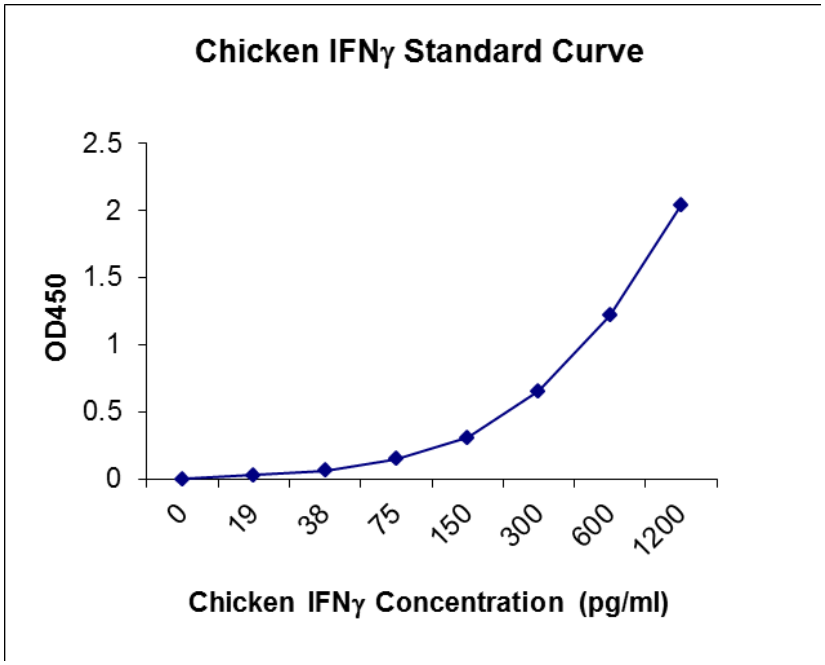
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create 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. The data may be linearized by plotting the log of the IFNγ 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.

The Standard Curve

The graph below represents typical data generated when using this Chicken IFNγ ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve.



Specificity

The following recombinant chicken proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, HGF, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, MMP-2, MMP-9, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, sIL2R, sIL6R, VEGF.

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant Chicken IFNγ.

Detection Range

19-1200 pg/ml

Assay Sensitivity

1 pg/ml

Assay Precision

Intra-Assay %CV: 7; Inter-Assay %CV: 9

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