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Chicken IFN Gamma ELISA Kit

Catalog number: NB-E60048 (96 wells)

The kit is designed to quantitatively detect the levels of Chicken IFN Gamma in ***cell culture supernatants, serum and plasma.***

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 20 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

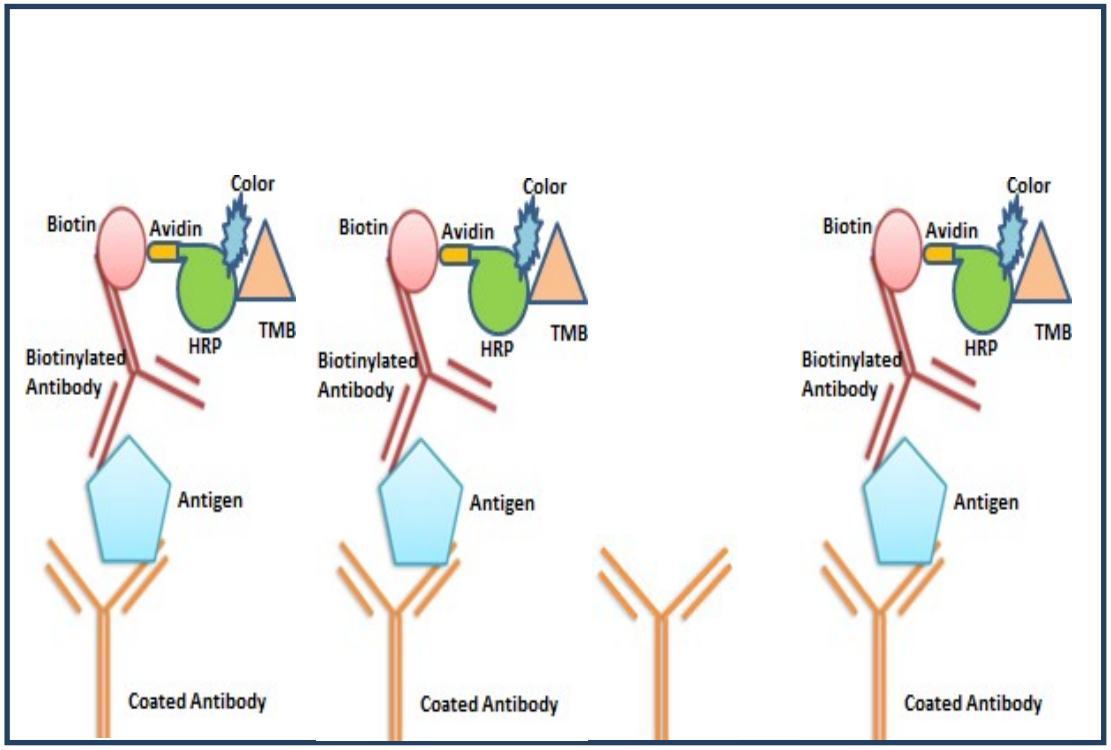
Intended use

The kit is used to quantify the Chicken IFN Gamma in cell culture supernatants, serum and plasma.

Standard range	15.6-1000 pg/ml
Sensitivity	1.0 pg/ml
Assay time	5 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Chicken IFN Gamma ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Chicken IFN Gamma specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated Chicken IFN Gamma specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the Chicken IFN Gamma amount of sample captured in plate.



Materials supplied

1.Chicken IFN Gamma standard:	5 ng/vialx2.
2. 96-well plate coated with anti-Chicken IFN Gamma Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Biotinylated Chicken IFN Gamma Ab:	1 vial.
5. Streptavidin-HRP:	1 vial.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. TMB developing reagent	12ml
9. Stop Solution:	6 ml.
10. 20 × Wash Buffer:	25 ml.
11. Plate sealer	1.
12. Package insert	1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.
- Heat inactivated normal goat serum (for detection antibody dilution)

Sample Preparation and storage

Cell culture supernatant: Remove particulates by centrifugation at 3000 x g for 10 minutes, analyze immediately or aliquot and store at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. **The user should determine the optimal dilution factor**.

Reagent Preparation

Standard

- Chicken IFN Gamma: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (5 ng/vial) are included in each kit. Use one tube for each experiment.

- 1000 pg/ml→15.6 pg/ml of Chicken IFN Gamma standard solutions:
- Add 1 ml of sample diluent into one standard tube with 5 ng Chicken IFN Gamma standard. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 5000 pg/ml standard solution.
- Label 7 Eppendorf tubes with 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.6 pg/ml, respectively. Aliquot 0.8 ml of sample diluent and 0.2 ml of 5,000 pg/ml standard solution into 1000 pg/ml tube, this is 1000 pg/ml standard solution. Then make 2-fold serial dilution from 1000 pg/ml to 15.6 pg/ml in seven 1.5 ml tubes.
- Make sure each tube has ≥ 300 µl of standard.

  Note: The standard solutions are best used within 2 hours.

Biotinylated Chicken IFN Gamma antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.
- Spin down before opening the vial. Biotinylated anti-Chicken IFN Gamma detection antibody should be diluted as instruction on the vial label with Antibody diluent buffer. Allow the diluted Detection Antibody to sit at least 1-2 hours before use.

Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).
- Spin down before opening the vial. Streptavidin-HRP should be diluted as instruction on the vial label with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Chicken IFN Gamma Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Chicken IFN Gamma concentration in samples.

1. Add 100 µl of sample or standards per well. Add 100 µl of the sample diluent into the control well (Zero well)., immediately Add 50 µl of biotinylated Chicken IFN Gamma antibody working solution to each well.. Cover with an adhesive strip and incubate 2 hours at room temperature. Note: We recommend that each Chicken IFN Gamma standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, 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 solution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
4. Repeat the aspiration/wash as in step 2 for five times.
5. Add 100 µl TMB developing reagent to each well. Cover and incubate for 10-15 minutes at room temperature (Protect from light. Do not over-develop).
6. Add 50 µl Stop Solution to each well. Mix well.
7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

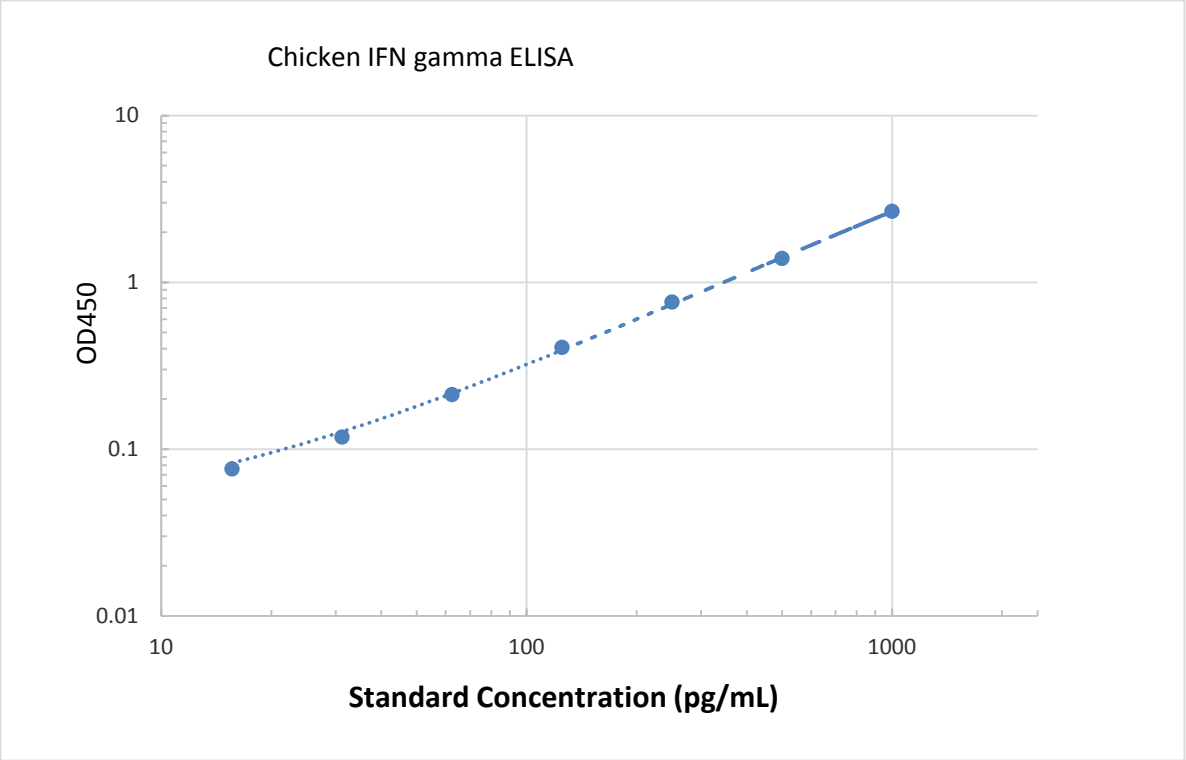
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Chicken IFN Gamma concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical data

This standard curve is for demonstration purpose only. A standard curve must be run with each assay.



Recovery

Matrices listed below were spiked with certain level of chicken IFN gamma and the recovery rates were calculated by comparing the measured value to the expected amount of IFN gamma in samples.

Matrix	Recovery range(%)	Average(%)
serum(n=5)	86-103	97
EDTA plasma(n=5)	87-99	92
heparin plasma(n=5)	88-97	93

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IFN gamma and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	86-104%	90-105%	89-100%	85-97%
EDTA plasma(n=5)	83-97%	87-97%	86-101%	84-101%

	heparin plasma(n=5)	82-100%	80-100%	86-100%	82-98%
CV(%)	Intra-Assay: CV<8% Inter-Assay: CV<10%				

Background

Interferon gamma (IFN-γ), also known as type II interferon, is a member of the cytokine family and the only member of the type II class of interferons identified so far. IFN-γ is produced by a number of cell types under inflammatory conditions, including dendritic epidermal/γδ T cells, keratinocytes, peripheral blood γδ T cells, mast cells, neurons, $CD8^+$ T cells, macrophages, B cells, neutrophils, NK cells, $CD4^+$ T cells, and testicular spermatids. Rat IFN-γ shares approximately 87% and 39% amino acid sequence identity with mouse IFN-γ and human IFN-γ, respectively. Consistent with their degrees of shared homology, rat IFN-γ is active on mouse cells but not on human cells. Biologically active IFN-γ consists of a noncovalently linked homodimer which binds to transmembrane IFN-γ RI (alpha subunit) coupled with transmembrane IFN-γ RII (beta subunit) to form a functional receptor complex of two α and two β subunits. IFN-γ is essential to promote anti-viral, anti-proliferative, and immunoregulatory activities. On many cell types, IFN-γ induces the production of cytokines and upregulates the expression of various membrane proteins including class I and II MHC antigens, Fc receptors, leukocyte adhesion molecules, and B7 family antigens. IFN-γ also plays a key role in the pathogenesis of certain inflammatory diseases such as autoimmunity and atherosclerosis.

Manufactured and Distributed by:

Novatein Biosciences

310 W Cummings Park

Woburn, MA 01801, USA

Phone: (617) 238-1396

Fax: (617) 380-0053

Toll Free: (888) 856-2858

http://www.novateinbio.com/

Email: Info@novateinbio.com