Bovine 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 ELISA kit is for quantification of IFNγ in bovine. 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 bovine 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 bovine 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 at 4°C. The kit should 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	20 x Wash Buffer	1	Stop Solution	1
Conjugate	1	10 x Reagent Diluent	1	Datasheet/Manual	1
Standard	3	20 x Standard/Sample Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Bovine IFNγ Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C in a manual defrost freezer for up to 3 months, if not used immediately. Spin to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS to the antibody vial, vortex 30 sec. Take the entire 200 µL of detection antibody to 10 mL of 1 x Reagent Diluent to make **working dilution of Detection Antibody** if the entire 96-well plate is used. If the partial antibody is used store the rest at -20° C until use.

Bovine IFNγ Standard (3 vials) –The lyophilized Bovine IFNγ Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The unreconstiuted standard can be stored at -20°C for up to 3 months if not used immediately. Spin to bring down the material prior to open the tube. Add 500 µL of 1 x Standard/Sample Diluent to a Standard vial to make the high standard concentration of 1,200 pg /ml. Vortex 30 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 the Standard/Sample Diluent, vortex 30 sec for each of dilution step.

Conjugate (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. The vial contains sufficient conjugate for a 96-well plate. If the volume is less than 50 µL, add 1 x PBS to reach 50 µL. Make 1:200 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Conjugate to 10 mL of the Reagent Diluent to make **working dilution of Conjugate** prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 3 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, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent -Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent, vortex for 1 min and allow it to sit for 15 min to completely dissolve. Store at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
20 x Standard/Sample Diluent, 10 mL- Dilute to 1 x Standard/Sample 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** (such as plasma or serum) or **standard** in 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 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 Conjugate** 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 20 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. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standard at each of dilution steps is critical to ensure a normal calibration curve.
3. If IFNγ exceeds the upper limit of detection, sample needs to be diluted with 1 x Standard/Sample Diluent. Dilution factor must be used for calculation of the analyte concentration.
4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
5. The Stop Solution is an acid solution, handle with caution.
6. This kit should not be used beyond the expiration date on the label.
7. 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.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

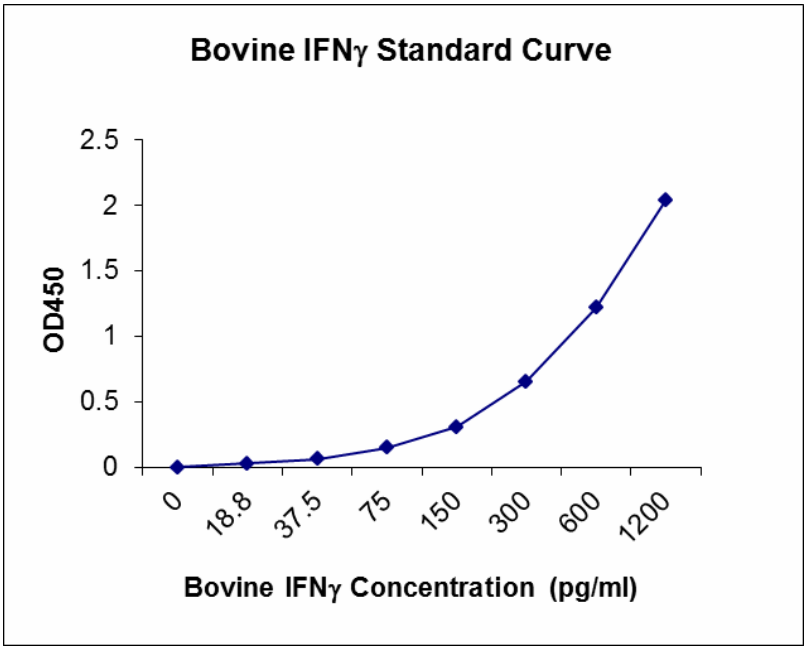
Calculation of Results

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 bovine 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. The correlation coefficient (r^2) is 0.999-1.0.



Specificity

The following recombinant bovine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
Adiponectin, ApoAI, BMP1, BMP2, BMP3, BMP4, BMP5, BMP7, CCL2, CCL4, CCL5, CRP, HGF, HSP27, IGF1, IL-β, IL-2, IL-4, IL-5, IL-6, IL-6R, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-21, IL-23, IFNβ1, MMP2, MMP9, PDGF, PLA2G7, serpin E1, TGFβ1, TGFβ2, TGFβ3,TLR1, TLR2, TLR3, TLR9, TNF-α, TNF RI, TNF RII, VEGF, VEGF R1.

Calibration

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

Detection Range

18-1200 pg/ml

Assay Sensitivity

4 pg/ml

Assay Precision

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

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