Bovine IFN-β ELISA Kit

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells, and are very important for fighting viral infections. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines and are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: activation of immune cells, such as natural killer cells and macrophages; increasing recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes; and increasing the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection. About ten distinct IFNs have been identified in mammals and are typically divided among three IFN classes: Type I, Type II and Type III IFN and IFN-β belongs to type I IFNs bind to a specific cell surface receptor complex known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains.[1]

In addition to the JAK-STAT pathway, IFNs can activate several other signaling cascades. Both type I and type II IFNs activate a member of the CRK family of adaptor proteins called CRKL, a nuclear adaptor for STAT5 that also regulates signaling through the C3G/Rap1 pathway.[2] Type I IFNs further activate *p38 mitogen-activated protein kinase* (MAP kinase) to induce gene transcription.[2] The immune effects of interferons have been exploited to treat several diseases. Interferon beta-1a and interferon beta-1b are used to treat and control multiple sclerosis, an autoimmune disorder. This treatment is effective for slowing disease progression and activity in relapsing-remitting multiple sclerosis and reducing attacks in secondary progressive multiple sclerosis.[3]

References

1. de Weerd NA, et al. (2007). *J Biol Chem* **282** (28): 20053–20057.
2. Platanias, L. C. (May 2005). *Nature reviews. Immunology* **5** (5): 375–386.
3. Paolicelli, D. (2009). *Biologics: Targets & Therapy* **3**: 369–376.

PRINCIPLE OF THE ASSAY

This kit is for quantification of IFNβ in pig. This is a shorter 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 IFNβ has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFNβ present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Bovine IFNβ 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 IFNβ 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 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	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 IFNβ Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 3 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 sterile 1 x PBS, vortex 20 sec and allow it to sit for 5 min prior to use. Take the entire 200 µL of detection antibody to 10 mL 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 IFNβ Standard (3 vials) – The lyophilized Bovine IFNβ 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 Standard/Sample Diluent to a Standard vial to make the high standard concentration of 1600 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 the Standard/Sample Diluent, vortex 20 sec for each of dilution steps.

Conjugate (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 50 µL conjugate sufficient for a 96-well plate. If the volume is less than 50 µL, add reagent diluent to final volume of 50 µL. Make 1:200 dilution in 1 x Reagent Diluent. If an entire 96-well plate is used, add 50 µL of Conjugate to 10 mL of 1 x Reagent Diluent to make **working dilutions of Conjugate** prior to the assay. The rest of undiluted Conjugate 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, 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 prior to use.
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 **2 hours** 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 **2 hours** 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 standards at each dilution step is crucial to ensure a normal standard curve.
3. If IFNβ exceeds the upper limit of the detection, the 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.

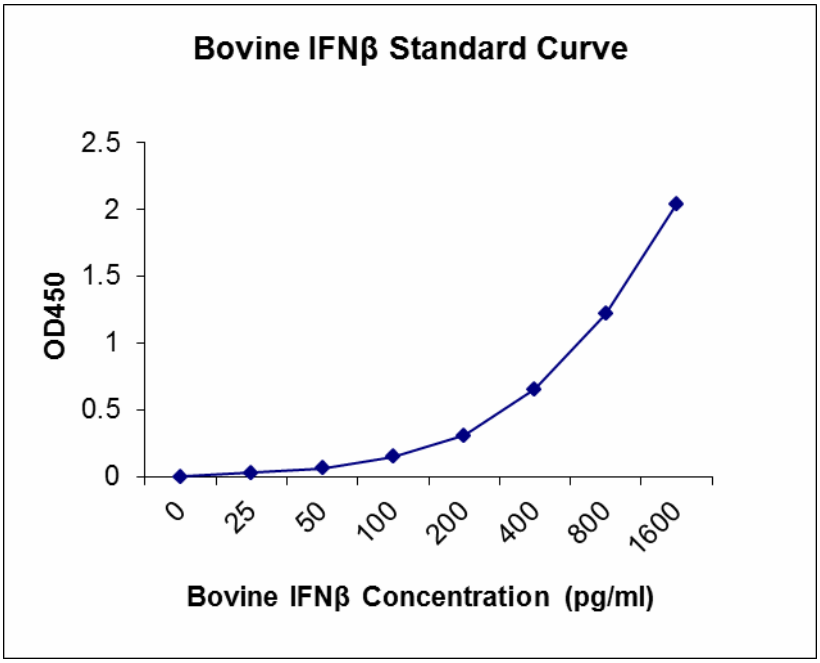
Calculation of Results

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 IFNβ 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 IFNβ 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 bovine 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 bovine IFNβ.

Detection Range

25-1600 pg/ml

Assay Sensitivity

5 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 9

For Research Use Only.

Related products

1. Bovine IFNβ Standard
2. Bovine IFNβ Detection Antibody