Porcine IFN-β Immunoassay

Catalog Number: SEKP-0046

For the quantitative determination of Porcine IFN-β concentrations in cell culture supernates, serum, and plasma.

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

MANUFACTURED AND DISTRIBUTED BY:

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TABLE OF CONTENTS

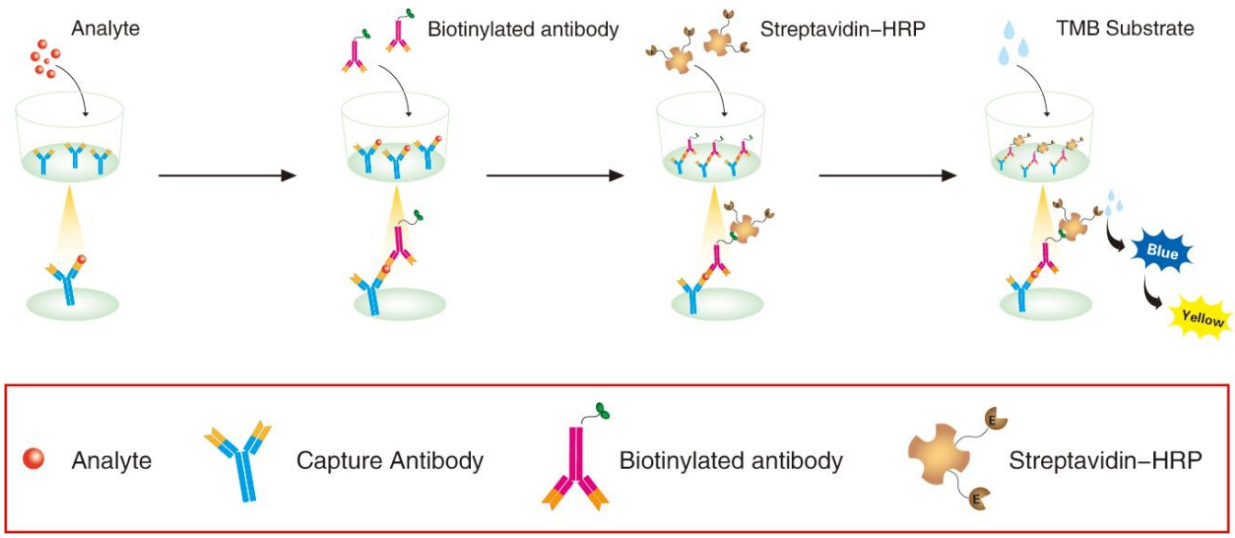
BACKGROUND

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.In addition to the JAKSTAT 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. Type I IFNs further activate *p38 mitogen-activated protein kinase* (MAP kinase) to induce gene transcription.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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IFN- β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN- β present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IFN- β is added to detect the captured IFN- β protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram：



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized, 2200 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vials	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C** for six months
Substrate Solution	1 bottle	Store at 2-8°C** for six months
Stop Solution	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

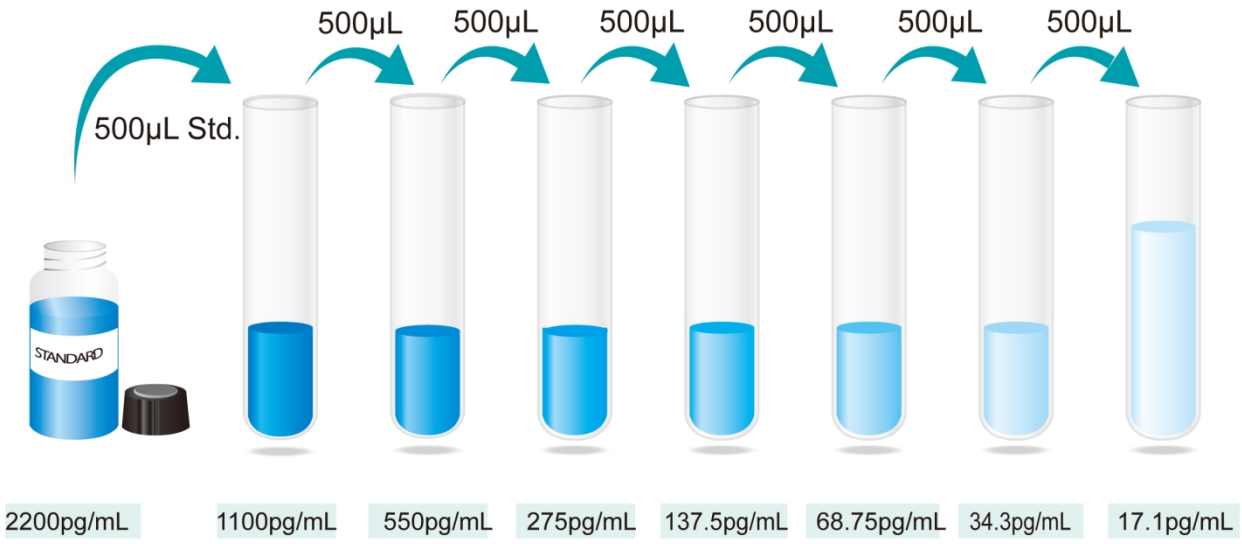
Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Plasma or serum sample should be diluted with equal volume of Standard/Sample diluent and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample(2 vials)** - Porcine IFN-β Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of **Standard /Sample Diluent**. This reconstitution produces a stock solution of 2200pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making

dilutions.Pipette 500µL of **Standard /Sample Diluent** into 1100pg/ml tube and the remaining tubes. Use the stock solution of 2200pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 2200pg/mL standard serves as the high standard. The **Standard /Sample Diluent** serves as the zero standard (0 pg/mL).



Preparation of Porcine IFN-β standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Porcine IFN-β antibody(1 vials)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP(120µL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 µL HRP Conjugate sufficient for a 96-well plate.Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add

100 ul of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4° C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

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Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩

Add 100µl working solution of porcine-Conjugate anti-Porcine IFN- β antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

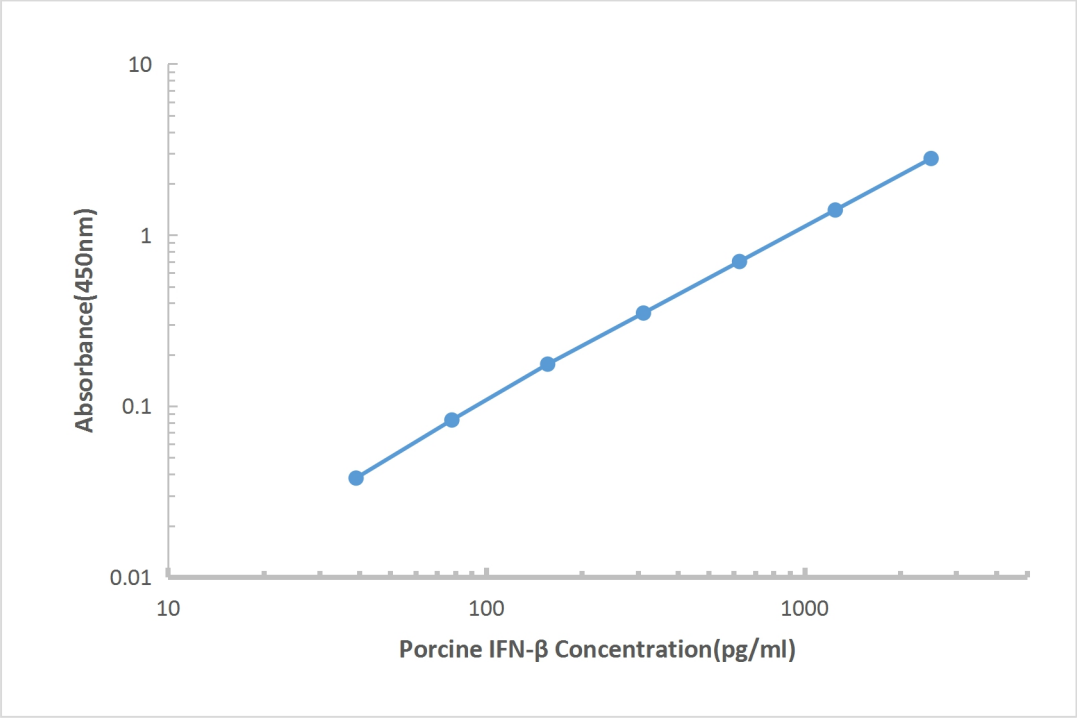
1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using 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.

4. The data may be linearized by plotting the log of the IFN- β 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.

5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IFN- β ELISA

Std (pg/mL)	O.D.1	O.D.2	Averag	Correct
0	0. 042	0. 041	0. 0415	---
17.18	0. 098	0. 097	0. 0975	0. 056
34.375	0. 129	0. 125	0. 127	0. 0855
68.75	0. 373	0. 358	0. 3655	0. 324
137.5	0. 698	0. 677	0. 6875	0. 646
275	1. 272	1. 293	1. 2825	1. 241
550	2. 051	2. 076	2. 0635	2. 022
1100	2. 753	2. 774	2. 7635	2. 722



Representative standard curve for IFN- β ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 7 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Porcine IFN-β. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

BMP1, BMP2, BMP3, BMP4, IL-2, IL-4, IL-5, IL-6, IFNγ, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α.

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REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of IFN-β spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IFN-β in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	97	89-104
Cell culture supernatants	96	87-107

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IFN-β in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	96	103
	Range (%)	89-103	96-113
1:4	Average% of Expected	98	105
	Range (%)	90-105	97-115

REFERENCES

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2. Platanias, L. C. (May 2005). *Nature reviews. Immunology* **5** (5): 375–386.
3. Paolicelli, D. (2009). *Biologics: Targets & Therapy* **3**: 369–376.