Sheep IFN beta ELISA Kit

Sheep IFN beta ELISA Kit is an Enzyme Immunoassay kit for the quantification of Sheep IFN beta in serum, plasma and cell culture supernatants.

Catalog number: ARG82814

Package: 96 wells

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INTRODUCTION

The IFN-β proteins are produced in large quantities by fibroblasts. They have antiviral activity that is involved mainly in innate immune response. Two types of IFN-β have been described, IFN-β1 (IFNB1) and IFN-β3 (IFNB3) (a gene designated IFN-β2 is actually IL-6). IFN-β1 is used as a treatment for multiple sclerosis as it reduces the relapse rate.

IFN-β1 is not an appropriate treatment for patients with progressive, non-relapsing forms of multiple sclerosis. [Provide by Wikipedia: Interferon beta]

PRINCIPLE OF THE ASSAY

This Sheep IFN beta ELISA kit is a quantitative sandwich enzyme immunoassay that measures the amount of Sheep IFN beta in the samples. An antibody specific for Sheep IFN beta has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN beta present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Sheep IFN beta 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 IFN beta bound in the initial step. The color development is stopped, and the intensity of the color is measured.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at 2-8°C upon receiving. Do not use kit components past kit expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
Standard	3 vials (lyophilized)	4°C
20X PBS	25 mL	4°C
20X Assay Buffer	20 mL	4°C
Detection Antibody	1 vial (lyophilized)	4°C
HRP Conjugate	55 µL	4°C
TMB Substrate	10.5 mL	4°C
Stop Solution	5.5 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X PBS / Assay Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles.

Cell culture supernatant: Centrifuge at 300 x g for 10 minutes at 4°C to remove the cell debris.

Note:

- Samples should be diluted with four volumes of 1 x Assay Buffer and vortex for 1 minute 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.
- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X PBS: Dilute 20X PBS into Distilled water to yield 1X PBS
  (E.g., add 30 mL of 20X PBS into 570 mL of distilled water to a final volume of 600 mL).
- 1X Assay Buffer: Dilute 20X Assay Buffer into 1X PBS to yield 1X Assay Buffer
  (E.g., add 20 mL of 20X Assay Buffer into 380 mL of 1X PBS to a final volume of 400 mL).
- Detection Antibody: The lyophilized Detection Antibody should be stored at 4°C for up to 6 months, if not used immediately. Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Add 200 µL of 1X PBS to the antibody vial, vortex 20 seconds and allow it to sit for 5 minutes. Then, take reconstituted 200 µL of detection antibody to 10.5 mL of 1X PBS to make working Detection Antibody and vortex 30 seconds. If the partial antibody is used store the rest at -20°C until use.
- HRP Conjugate: Centrifuge at 3000 x g for 1 minute to bring down the material prior to open the vial. Make 1:200 dilution with 1X PBS. Then, take diluted 55 µL of HRP Conjugate into 10.5 mL of 1X PBS to make working HRP Conjugate and vortex 30 sec prior to the assay. The rest of undiluted HRP Conjugate can be stored at 2°C- 8°C for up to 3 months. DO NOT FREEZE.

- **Standard:** The un-reconstituted standard can be stored at 4°C for up to 3 months if not used immediately. Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Add **500 µL** of **1X Assay Buffer** to a Standard vial to make the high standard concentration of **1600 pg/ml** and vortex 1 minute and allow it to sit for 5 minutes. Diluted the standard as follow.

Standard tube	IFN beta (pg/mL)	1X Assay Buffer (µL)	Standard stock, 1600 pg/mL (µL)
S1	1600	0	500
S2	800	250	250 of S1
S3	400	250	250 of S2
S4	200	250	250 of S3
S5	100	250	250 of S4
S6	50	250	250 of S5
S7	25	250	250 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. It is recommended that all samples and standards be assayed in duplicate.

1. Add **100 µL** of **samples** and **Standard** into respective wells.

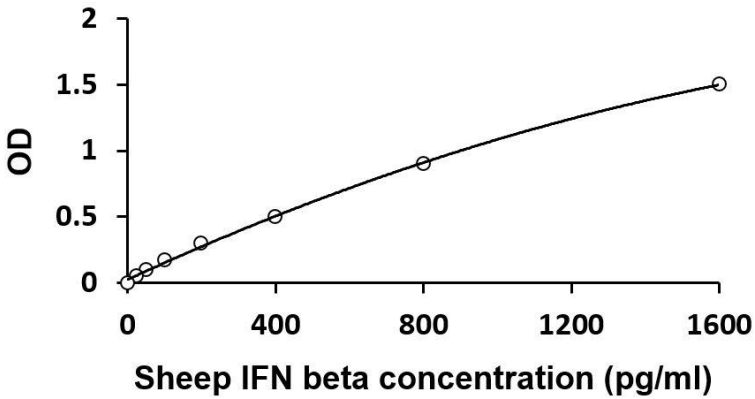
   Note: Must vortex standards and samples for 10 sec before pipetting to the wells.
2. Cover the plate and incubate for **1.5 hour** at **room temperature**.
3. Aspirate each well and wash, repeating the process 1 time for a total 2 washes. Wash by filling each well with **1X Assay Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **Working Detection Antibody** to each well. Mix well by repeated pipetting.
5. Cover the plate and incubate for **1.5 hour** at **room temperature**.
6. Aspirate and wash plate as in step 3.
7. Add **100 µL** of **Working HRP Conjugate** to each well.
8. Cover the plate and incubate for **20 mins** at **room temperature** in the dark.
9. Aspirate and wash plate as in step 3, but wash for **4 times washes**.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **5-30 mins** at **room temperature** in the dark.
12. Add **50 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.**

CALCULATION OF RESULTS

1. Subtract zero point (S0) from all standards and unknowns to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Guinea Pig IFN beta ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

12.5 pg/mL

Precision

Intra Assay CV: 6.0%; Inter Assay CV: 9.0%