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Human IFN-beta ELISA Kit

Catalog No: CKH324 / CKH325 **Quantity:** 1 x 96 tests / 5 x 96 tests

Assay Range: 25-2000 pg/ml

Specifications:
This kit quantitates human interferon beta in media using a sandwich immunoassay. The kit is based on an ELISA with streptavidin conjugated to horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. The assay is based on the international reference standard for human interferon beta (Hu-IFN-) provided by the National Institutes of Health.

Speed: Incubation time, 3 hr 15 min

Specificity: Human IFN-b. No cross reactivity with human IFN-a, human IFN-g, mouse IFN-b or rat IFN-b.

Storage Conditions: For retention of activity, all reagents except the IFN standard should be kept at 2-4°C in the dark. The IFN standard should be kept at -20°C for one time use only.
Please note that the dilutions of the Detection Antibody and HRP Conjugate Concentrate differ from lot to lot as a result of calibrating each kit for optimal sensitivity. Please refer to the lot specific Certificate of Analysis (COA) for their preparation.
CAUTION: Solutions A, C, and F contain 0.1% Kathon CG/ICP as a preservative; they should be handled with appropriate safety precautions and discarded properly. For further information, consult the material safety data sheet for Kathon CG/ICP.

Introduction: Interferon beta (IFN-b) is synthesized and secreted by fibroblasts and a many other cell types in response to pathogens. These include viruses and bacteria and can occur through Toll-like receptor dependent or independent pathways. Following secretion, IFN-b binds to type I interferon receptors on proximal or distal cells activating the JAK1-STAT signaling pathway. Activation of this signal transduction pathway leads to the expression of 2q5q oligoadenylate synthetases (2q5qOAS), protein kinase R (PKR), MxA proteins, and interferon regulatory factor 7 (IRF-7). The upregulation of IRF-7 expression can exert a positive feedback on IFN-b production, whereas the induction of 2q5qOAS activates a latent endonuclease known as RNase L. RNase L cleaves both viral and cellular single stranded mRNA, thereby limiting viral replication and dissemination.

Materials Provided:
Pre-coated microtiter plate(s)
Plate sealers
A: Wash Solution Concentrate
B: Human IFN Standard 100,000 pg/ml (stock solution)
C: Sample Diluent
D: Antibody Concentrate
E: HRP Conjugate Concentrate
F: Concentrate Diluent
G: TMB Substrate Solution
H: Stop Solution

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Additional Materials Required:

˝ Microtiter plate reader capable of reading a wavelength of 450nm
˝ Variable volume micro-titer pipettes
˝ Adjustable multi-channel pipette (50-200 ml)
˝ Reagent reservoirs
˝ Wash bottle or plate washing system
˝ Distilled or deionized water
˝ Serological pipettes (1, 5, 10 or 25 ml)
˝ Disposable pipette tips (polypropylene)

ELISA Procedure:

Preparation of Reagents:

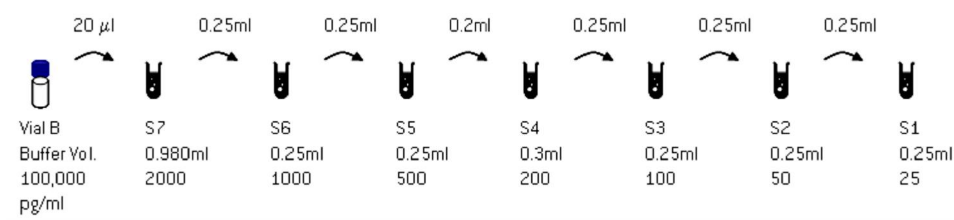
Wash Buffer: Dilute 50 ml of the Wash Solution Concentrate to a final volume of 500 ml with distilled or deionized water. Mix thoroughly before use. The diluted wash buffer can be stored at (2-25°C).
Human IFN Beta Solution: Dilute the Human Interferon Beta Standard, provided at 100,000 pg/ml, in Sample Diluent as indicated. In certain situations %test+samples may contain substances that can interfere with assay results. Therefore, it is recommended to run the IFN standard curve diluted in your sample matrix.
Construct a standard curve from 2000-25 pg/ml:
a. Label seven polypropylene tubes (S7-S1).

b. Fill tubes with Sample Diluent as indicated.

c. Using polypropylene tips add the working stock of Human IFN Beta Standard to S7 and mix gently. Change tips between each dilution.

d. Remove indicated amount from S7 and add to S6. Repeat to complete series to S1.

e. Refrigerate until use in step 1 of the assay procedure.



Sample Preparation: Prepare test samples of unknown interferon concentration to be tested using Sample Diluent as required. Measurements in duplicate are recommended. Refrigerate until use in step 1 of the assay procedure.

Antibody Solution: Dilute Antibody Concentrate in Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amounts of Antibody Solution to prepare. Refrigerate until use in step 2 of the assay procedure.

HRP Solution: Dilute HRP Conjugate Concentrate in Concentrate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amounts of HRP Solution to prepare. Refrigerate until use in step 3 of the assay procedure.

Assay Procedure - Quick Reference

Step	Reagent	Volume/ well	Incubation	Wash	Comments
1	Standard and Samples	100 µl	60 min	3X	Include blanks containing Sample Diluent only
2	Diluted Antibody Solution	100 µl	60 min	3X	
3	Diluted HRP Solution	100 µl	60 min	3X	During incubation, **warm TMB to room temp**.
4	TMB Substrate	100 µl	15 min	DO NOT WASH	Incubate in the dark; no plate sealer
5	Stop Solution	100 µl	0 min	DO NOT WASH	Read within 5 minutes
6	Read Plate at 450nm				

Note: All incubations are at room temperature

Assay Procedure – Detailed

All incubations should be performed in a closed chamber at 24°C or alternatively at room temperature (22-25°C) keeping the plate away from drafts and other temperature fluctuations. Use plate sealers to cover the plate as directed. During all wash steps remove contents of plate by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry. All wells should be filled with a minimum of 250 µml of diluted wash solution. Refer to preparation of reagents for diluted solutions.

1. Standards and Samples: Determine the number of microwell strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. Each standard, blank and sample should be run in duplicate. We recommend using strips 1 and 2, rows A-H for serially diluted standards and blanks. Remove extra microtiter strips from the frame, seal in the foil bag provided and store at 2-4°C. Unused strips can be used in later assays. Add 100 l per well of the interferon standards, blanks and samples. Cover and incubate for 1 hour.

After 1hour, empty the contents of the plate and wash the wells three times with diluted wash buffer (Refer to preparation of reagents).

2. Antibody: Add 100 l of diluted antibody solution to all wells. Cover and incubate for 1 hour.
After 1 hour, empty the contents of the plate and wash the wells three times with diluted wash buffer.

3. HRP: Add 100 l of diluted HRP solution (refer to preparation of reagents) to all wells. Cover and incubate for 1 hour. During this incubation period, warm the TMB Substrate Solution to room temperature (22-25°C).

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After 1 hour, empty the contents of the plate and wash the wells three times with diluted wash buffer.

4. TMB Substrate: Add 100 l of the TMB Substrate Solution to each well. Incubate, in the dark, for 15 minutes. Do not use a plate sealer during the incubation.

5. Stop Solution: After the 15 minute incubation of TMB, DO NOT WASH. Add 100 ml Stop Solution to each well.

6. Read: Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of the Stop Solution.

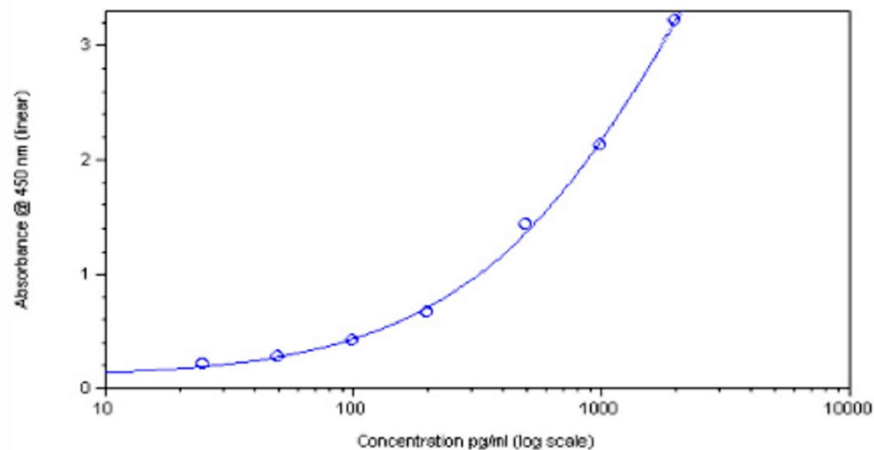
Calculation of Results

By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Blank ODqs should be subtracted from the standards and sample ODqs to eliminate background.

Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 10. 25 pg/unit is applicable for human interferon beta. Nevertheless, this conversion factor is only an approximation.

A shift in optical densities is typical between users and kit lots. The back fit concentration extrapolated from the standard curve is a more accurate determination of the sample titer and performance of the kit. Variations, from the typical curve provided can be a result of operator technique, altered incubation time, fluctuations in temperature, and kit age.

Results of a typical standard graph using a 4-parameter fit are provided for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.



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