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**Human Interferon Omega
 (Hu-IFN- ω) ELISA Kit**
 v.1.3

Product #41395-1
 Single Plate (96 Tests)

Lot Number:3167

Expiration Date: 31 August 2007

Sensitivity Range: 5 – 300 pg/ml

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Please review the protocol in its entirety prior to use to insure proper kit performance.

Speed: Incubation time, 3 hr 15 min

Specificity: Human IFN- ω . No cross reactivity with human IFN- γ , human IFN- β or human IFN- α .

Specifications: This kit quantitates human interferon omega in cell culture supernatants, human serum, plasma, or other body fluids using a sandwich immunoassay.^{1,2} The kit is an ELISA that uses biotinylated antibody and streptavidin conjugated horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. All reagents are supplied. Two pre-coated microtiter strip plates (96 wells) are included. Typical standard curves for each lot are included with the procedure.

Special Conditions/Comments: For retention of activity, all reagents should be kept at 2-8°C in the dark. Deionized or distilled water should be used for preparation of all reagents. All dilutions should be made with polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution tube. All measurements for standards and samples should be performed in duplicate. At least two control wells (wells with Dilution Buffer only) should be used for each assay; these control values should be subtracted from all readings prior to any calculations or plots of the data.

Specimen Collection: Cell culture supernatants, human serum, EDTA, heparin, and citrate plasma or other biological samples will be suitable use in the assay. Remove serum from the clot or red blood cells, respectively, as soon as possible after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or limedic specimens. Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20oC to avoid loss of bioactive IFN- ω . If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

Materials Supplied:

Kit Components			#41395-1
Size (plate)	Lot#	Volume	
Plate (s)	10568	1	
Plate Sealers	n/a	4	
Biotin – Conjugate*	16771	200 μ l: anti-IFN- ω monoclonal (murine) antibody	
Streptavidin-HRP*	17451	150 μ l	
IFN- ω Standard Concentrate*	10622	6000pg/mL [2 x 50 μ l]	
Wash Buffer Concentrate	16489	50ml	
Assay Buffer Concentrate*	15701	5ml	
Sample Diluent	16378	12ml	
Substrate Solution I	16489	7ml	
Substrate Solution II	15701	7ml	
Stop Solution	17404	12ml	
Blue-Dye	18313	0.4ml	
Green-Dye	18413	0.4ml	
Red-Dye	17993	0.4ml	

* Reagents contain preservatives

Preparation of Reagents

A. **Wash Buffer:** If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved. Pour entire contents (50ml) of the *Wash Buffer Concentrate* into a clean 1,000-ml graduated cylinder. Bring final volume to 1,000ml with glass distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer may be prepared as needed according to the following table:

Number of strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1-6	25	475
1-12	50	950

B. **Assay Buffer:** Mix the contents of the bottle well. Add contents of *Assay Buffer Concentrate* (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1-6	2.5	47.5
1-12	5.0	95.0

C. **Preparation of Biotin-Conjugate:** Make a 1:100 dilution of the Biotin-Conjugate with *Assay Buffer* (reagent B) in a clean plastic tube as needed according to the following table:

5 ml Diluent	20 μ l <i>Blue-Dye</i>
12 ml Diluent	48 μ l <i>Blue-Dye</i>

Number of strips	Biotin – Conjugate (ml)	Assay Buffer (ml)
1-6	0.03	2.97
1-12	0.06	5.94

D. **Preparation of IFN- ω Standard:** Add 450 μ l of *Assay Buffer* to one vial of concentrated **IFN- ω Standard** as needed. Shake gently to mix. Store diluted Standard promptly at -20°C after use.

E. **Preparation of Streptavidin-HRP:** a) Make a 1:300 dilution with *Assay Buffer* (reagent B) of the concentrated *Streptavidin-HRP* solution as needed according to the following table:

Number of strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1-6	0.020	6
1-12	0.040	12

b) Alternatively, the *Streptavidin-HRP concentrate* may be prediluted by adding 300 μ l of *Assay Buffer* (reagent B) to the vial containing the concentrate. Mix the contents of the tube well. Make a further 1:100 dilution of this solution, as needed, according to the following table:

Number of strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1-6	0.060	6
1-12	0.120	12

F. **TMB Substrate Solution:** Using clean pipettes and containers known to be metal free, dispense an equal volume of *Substrate Solution I* into *Substrate Solution II* and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue color present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation by assay size:

Number of strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1-6	3.0	3.0
1-12	6.0	6.0

G. **Addition of color-giving reagents: Blue-Dye, Green-Dye, Red-Dye:** In order to help our customers avoid any mistakes in pipetting the ELISAs, PBL now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure. This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test. However, it can also be omitted by skipping to the Test Protocol section. The dye solutions from the stocks provided (*Blue-Dye, Green-Dye, Red-Dye*) can be added to the reagents according to the following guidelines:

1. **Diluent:** Before sample dilution, add the *Blue-Dye* at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol.

2. **Biotin-Conjugate:** Before dilution of the concentrated conjugate, add the *Green-Dye* at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of *Green-Dye* according to step C. Preparation of Biotin-conjugate.

3 ml Assay Buffer	30µl <i>Green-Dye</i>
6 ml Assay Buffer	60µl <i>Green-Dye</i>
12 ml Assay Buffer	120µl <i>Green-Dye</i>

3. **Streptavidin-HRP:** Before dilution of the concentrated Streptavidin-HRP; add the *Red-Dye* at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of *Red-Dye* according to step E Preparation of Streptavidin-HRP.

6 ml Assay Buffer	24µl <i>Red-Dye</i>
12 ml Assay Buffer	48µl <i>Red-Dye</i>

Test Protocol

- a. Mix all reagents thoroughly without foaming before use.
- b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human IFN- ω from holder and store in a foil bag with the desiccant provided at 2° - 8°C sealed tightly.
- c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
- After the last wash, empty wells and tap microwell strips on absorbent pad or paper towels to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.
- d. Add 100 µl of **Sample Diluent**, in duplicate, to all standard wells. Prepare standard dilutions by pipetting 100 µl of diluted (Refer to preparation of reagents) **IFN- ω Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of IFN- ω standard dilutions ranging from 300 to 4.7 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.
- e. Add 100 µl of **Sample Diluent**, in duplicate, to the blank wells.
- f. Add 75 µl of **Sample Diluent** to all wells designated as samples.
- g. Add 25 µl of each **Sample**, in duplicate, to the designated wells and mix contents.
- h. Prepare **Biotin-Conjugate**. (Refer to preparation of reagents)
- i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.
- j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours on a rotator set at 100 rpm, if available.
- k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.
- l. Prepare **Streptavidin-HRP**. (Refer to preparation of reagents)

- m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
- n. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour, if available on a rotator set at 100 rpm.
- o. Prepare **TMB Substrate Solution** a few minutes prior to use. (Refer to preparation of reagents).
- p. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.
- q. Pipette 100 µl of mixed **TMB Substrate Solution** to all wells, including the blank wells.
- r. Incubate the microwell strips at room temperature (18° to 25°C) for about 5 to 10 minutes on a rotator set at 100 rpm, if available. Avoid direct exposure to intense light. The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 OD. Therefore the color development within individual microwells must be watched by the person running the assay and the substrate reaction stopped before positive wells are no longer properly recordable.
- s. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
- t. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length: 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the IFN- ω standards.

Note: In case of incubation without shaking, the obtained O.D. values may be lower, but the results are still valid.

Calculation of Results

Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean.

Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the IFN- ω concentration on the abscissa. Draw a best-fit curve through the points of the graph.

To determine the concentration of circulating IFN- ω for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding IFN- ω concentration.

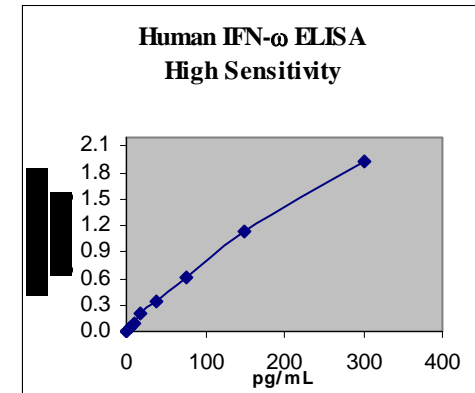
For samples which have been diluted according to the instructions given in this manual (1-4), the concentration read from the standard curve must be multiplied by the dilution factor (x 4).

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low IFN- ω levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual IFN- ω level.

It is suggested that each testing facility establishes a control sample of known IFN- ω concentration and runs this additional control with each assay. If the

values obtained are not within the expected range of this control, the assay results may be invalid.

A representative standard curve for Human IFN- ω ELISA is provided below. This curve is to be used for demonstration only and cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.



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