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QuickDetect™ IFN-alpha (Human) ELISA Kit

rev 02/21

(Catalog # E4450-100, 100 assays, Store at 4°C)

I. Introduction:

Human type I interferons (IFNs) including IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω are a large subgroup of interferon proteins that help regulate the activity of the immune system. The IFN-α proteins are produced by leukocytes. They are mainly involved in innate immune response against viral infection. All type I IFNs bind to a specific cell surface receptor complex known as the IFN-α/β receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. Once released, type I interferons will activate molecules which prevent the virus from producing and replicating its RNA and DNA. Overall, IFN-α can be used to treat hepatitis B and C infections. BioVision's IFN-alpha ELISA kit is a sandwich ELISA assay for the quantitative measurement of IFN-alpha in human serum, plasma and cell culture supernatants in 90 minutes. The density of color is proportional to the amount of IFN-alpha captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of IFN-alpha.
Detection Range: 0.25 - 32 pg/ml
Sensitivity: < 0.1 pg/ml
Assay Precision: Intra-Assay: CV < 10%; Inter-Assay: CV < 12% (CV (%) = SD/mean X 100)

III. Specificity:

Human

IV. Sample Type:

Serum, plasma, urine, cell culture samples, biological fluid

V. Kit Contents:

Components	E4450-100	Part No.
Micro ELISA strip-plate	1	E4450-100-1
Standard (40 pg/ml)	0.5 ml	E4450-100-2
Standard diluent	6 ml	E4450-100-3
HRP- Conjugate reagent	10 ml	E4450-100-4
Sample diluent	6 ml	E4450-100-5
Chromogen Solution A	6 ml	E4450-100-6
Chromogen Solution B	6 ml	E4450-100-7
Stop Solution	6 ml	E4450-100-8
Wash buffer (20X)	25 ml	E4450-100-9
Plate sealers	2	E4450-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer**: Dilute the concentrated washing buffer (20X) with distilled water.
2. **Standard Preparation:**

Ten wells are set for standards in the Micro ELISA strip-plate. In Well 1 and Well 2, 50 µl Standard solution and 50 µl Standard Dilution buffer are both added and mixed in each well. In Well 3 and Well 4, 50 µl solution from Well 1 and Well 2 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells is 50 µl and the concentrations are 20 pg/ml, 10 pg/ml, 5 pg/ml, 2.5 pg/ml and 1.25 pg/ml respectively.

3. **Sample Preparation:**
   Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN_3 should be avoided for this assay.

- **Serum**: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuge again.
- **Plasma**: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuge again.
- **Urine**: Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.
- **Cell Samples**: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again.
- **Tissue Samples**: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. In sample wells, add 40 µl Sample dilution buffer and 10 µl samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Add HRP-Conjugate reagent 100 µl to each well, except blank well. Incubate 60 min at 37°C after sealed with plate sealer.
4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
6. Add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
7. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

X. CALCULATION:

Known concentrations of Human INF-alpha Standard and its corresponding reading OD is plotted respectively. The concentration of INF-alpha in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

XI. RELATED PRODUCTS:

- IFN-γ (human) ELISA Kit (Cat. No. K4773)
- IFN-gamma (mouse) ELISA Kit (Cat. No. K4117)
- Human CellExp™ IFN-alpha 2b, Human Recombinant (Cat No. 6459)
- IFN-alpha 2a Antibody (Cat. No. 6681)