PRODUCT INFORMATION & MANUAL

Human IFN-α Instant ELISA

BMS216INST

Enzyme-linked immunosorbent assay for quantitative detection of human IFN-α.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests

eBioscience
An Affymetrix Company

Human IFN-α Instant ELISA

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1 Intended Use

The human IFN-α Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IFN-α. **The human IFN-α Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

The interferons represent proteins with antiviral activity secreted from cells in response to a variety of stimuli. In mammals, class I interferon (IFN) genes form a superfamily consisting of three gene families, the alpha interferon (IFN-α), the beta interferon (IFN-β) and the interferon omega (IFN-ω) genes (1). In humans the INF-α family comprises more than 20 genes and pseudogenes giving rise to 15 different functional gene products. The various species of human IFN-α are closely related in amino acid sequences with homologies in the range of 80 to 100 %. The molecular weight of the recombinant human IFN-α species is about 19 kDa consisting of 166 (165 for IFN-α2) amino acid residues lacking any N-glycosylation (α14 has N-glycosylation). The cystin mediated disulphide bonds are essential for the biological activity of IFN-α. The secondary structure of IFN-α was determined to be mainly α-helical. Target analysis of human IFN-α suggests that the functional unit is a monomer. The genes coding for all known class I interferons have been located to chromosome 9, the coding sequences (cDNAs) are subcloned and characterized. High level expression of the interferons was achieved in E. coli giving rise to a protein essentially identical to the natural protein.
The interferons exhibit a huge number of biological effects. The antiviral activity led to the name interferon and serves to define the unit of interferon activity. On purification of the natural human leukocyte interferons (IFN-α), it was found that all fractions that exhibited antiviral activity also exhibited anti-growth activity. This observation was confirmed with purified recombinant interferons and extended to other activities like: stimulation of cytotoxic activities of lymphocytes and macrophages, natural killer cell activity as well as increase in expression of some tumor-associated antigens.
The antiproliferative and antitumor activities of interferon have led to the application as an antitumor agent. The interferons also modulate cellular differentiation.

A major effect of interferons is their modulation of antigens of the major histocompatibility complex (MHC). All interferons induce an increase in surface expression of class I MHC antigens. Expression of the Fc receptors is also stimulated by interferon. Alterations in surface antigens may be an important mechanism by which interferon can modulate cellular interactions.The interaction of the interferons with their receptors determines the biochemical events and their modulation of cellular functions. This is a complex process just in the beginning to be dissected.
The role of IFN-α as a disease marker and marker for immunotherapeutic approaches has been demonstrated for a number of different indications and pathological situations:

- During the acute phase of a viral infection IFN-α levels are significantly elevated in the majority of patients. IFN-α levels fall significantly during the period of convalescence at the time viral infection is indicated by seroconversion tests.

- Increased levels of IFN-α were found in the majority of patients suffering from inflammatory arthropathies like juvenile polyarthritis, rheumatoid arthritis, ancylosing spondylitis, polychondritis, psoriatic arthritis, polymyalgia rheumatica and sclerodermia. Elevated IFN-α levels have also been shown for other autoimmune disorders like systemic lupus erythematosus and systemic vasculitis.

- Serum IFN-α levels can help to distinguish between children with non-specific abdominal pain or mesenteric adenitis, and those with acute appendicitis.

- For resistant local recurrence in e.g. breast cancer and metastatic spread local infiltration of IFN-α is a new interesting approach. Intrapleurally administered interferon causes measurable serum concentrations which correlated with the degeneration of malignant cells.

The IFN-α product is manufactured and sold in the United States under license from Pestka Biomedical Laboratories, Inc. (d/b/a PBL InterferonSource) solely for research use in the form in which it is originally manufactured, packaged and sold. Any modification,

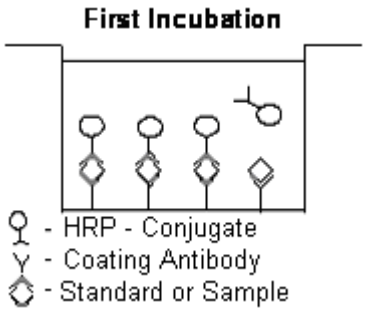
repackaging, or alteration of the product, and any use for diagnostic, therapeutic or clinical purposes is strictly prohibited.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

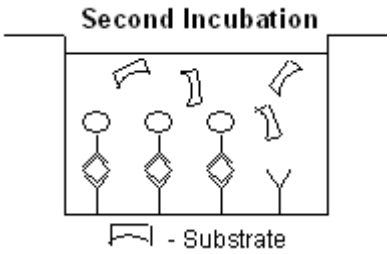
An anti-human IFN-α coating antibody is adsorbed onto microwells. Human IFN-α present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated anti-human IFN-α antibody binds to human IFN-α captured by the first antibody.

Figure 1



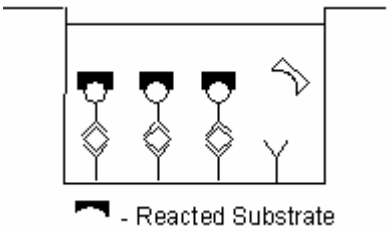
Following incubation unbound enzyme conjugated anti-human IFN-α is removed during a wash step and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human IFN-α present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IFN-α standard dilutions and human IFN-α sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human IFN-α, **HRP-Conjugate** (human IFN-α monoclonal antibody) and Assay Buffer, lyophilized

2 aluminium pouches with a human IFN-α **Standard curve** (coloured)

1 bottle (25 ml) **Wash Buffer Concentrate** 20x (phosphate-buffered saline with 1% Tween 20)

1 vial (5 ml) **Assay Buffer Concentrate** 20x (Use when an external predilution of the samples is needed)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum and plasma (EDTA, citrate, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells 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 lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human IFN-α. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 µl and 500 µl) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

The **buffer concentrates** should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in **buffer concentrates**, warm them gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

9.2 Assay Buffer (1x)

Pour the entire contents (5ml) of the Assay Buffer Concentrate (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
- **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
- **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
- **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
- **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
- **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**

a. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

c. Add 130 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (2500 pg/ml)	Standard 1 (2500 pg/ml)	Sample 1	Sample 1
B	Standard 2 (1250 pg/ml)	Standard 2 (1250 pg/ml)	Sample 2	Sample 2
C	Standard 3 (625 pg/ml)	Standard 3 (625 pg/ml)	Sample 3	Sample 3
D	Standard 4 (313 pg/ml)	Standard 4 (313 pg/ml)	Sample 4	Sample 4
E	Standard 5 (157 pg/ml)	Standard 5 (157 pg/ml)	Sample 5	Sample 5
F	Standard 6 (78 pg/ml)	Standard 6 (78 pg/ml)	Sample 6	Sample 6
G	Standard 7 (39 pg/ml)	Standard 7 (39 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 20 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.

e. Cover with an adhesive film and incubate at room temperature (18ºC to 25ºC) for 2 hours, if available on a microplate shaker at 400 rpm.

f. Remove adhesive film and empty wells. **Wash** the microwell strips 3 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash, tap microwell strips on absorbent pad or paper towel 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.

g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

   It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

i. 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.

j. 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 human IFN-α standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IFN-α concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human IFN-α 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 human IFN-α concentration.
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IFN-α levels. Such samples require further external predilution according to expected human IFN-α values with Assay Buffer (1x) in order to precisely quantitate the actual human IFN-α level.**
- It is suggested that each testing facility establishes a control sample of known human IFN-α concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 4

Representative standard curve for human IFN-α Instant ELISA. Human IFN-α was diluted in serial 2-fold steps in Assay Buffer. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

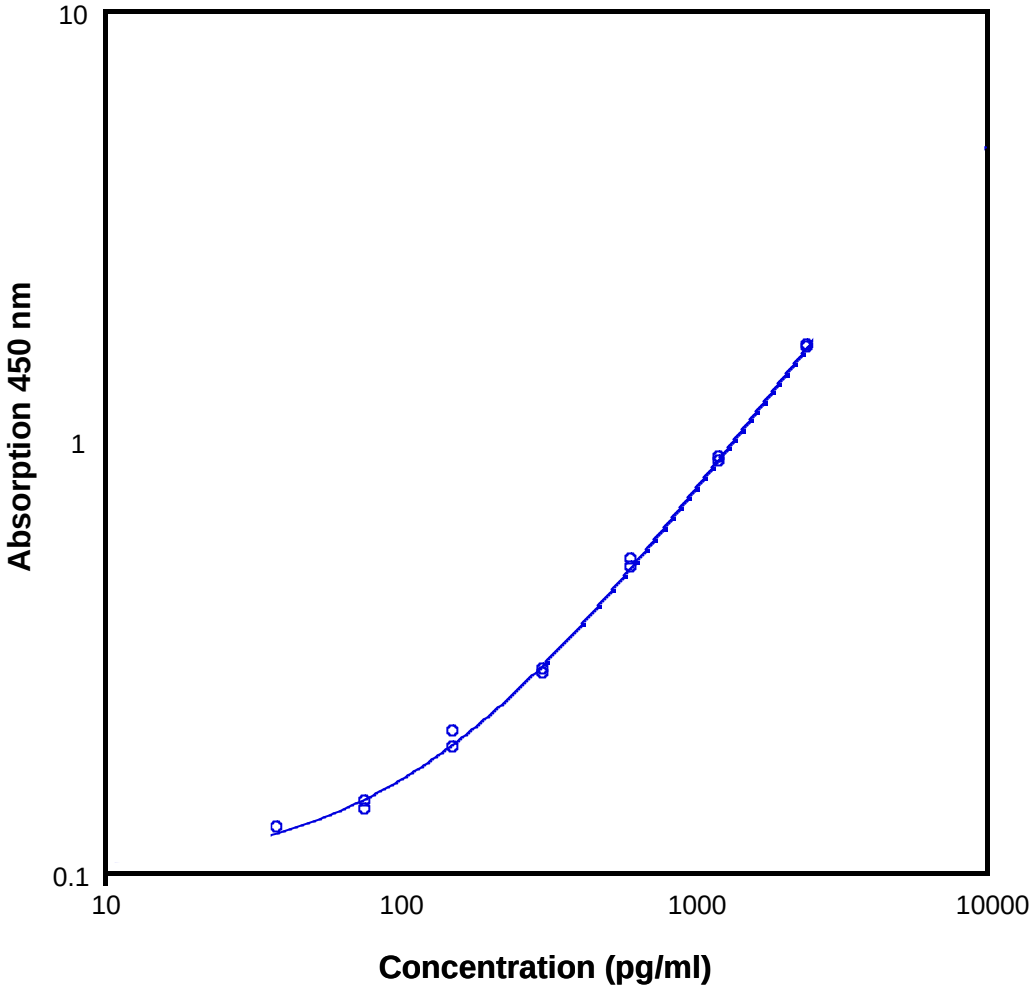


Table 2

Typical data using the human IFN-α INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human IFN-α Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	2500	1.650	1.660	0.8
		1.669		
2	1250	0.918	0.908	1.6
		0.898		
3	625	0.528	0.518	2.7
		0.508		
4	313	0.289	0.292	1.2
		0.294		
5	157	0.193	0.202	6.3
		0.211		
6	78	0.140	0.143	3.0
		0.146		
7	39	0.127	0.127	0.6
		0.126		
Blank	0	0.085	0.083	3.0
		0.080		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human IFN-α defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 3.3 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IFN-α. 2 standard curves were run on each plate. Data below show the mean human IFN-α concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.0%.

Table 3

The mean human IFN-α concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human IFN-α Concentration (pg/ml)	Coefficient of Variation (%)
1	1	1330	6
	2	1367	5
	3	1394	10
2	1	511	5
	2	494	5
	3	525	4
3	1	261	6
	2	250	9
	3	315	6
4	1	442	7
	2	418	6
	3	456	3
5	1	289	8
	2	274	3
	3	246	8
6	1	879	4
	2	873	6
	3	679	6

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IFN-α. 2 standard curves were run on each plate. Data below show the mean human IFN-α concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 7.4%.

Table 4
The mean human IFN-α concentration and the coefficient of variation of each sample

Sample	Mean Human IFN-α Concentration (pg/ml)	Coefficient of Variation (%)
1	1364	2.4
2	510	3.1
3	276	12.7
4	439	4.4
5	270	8.0
6	810	14.1

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IFN-α into human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Average recovery ranged from 80% to 104% with an overall mean recovery of 85%.

13.4 Dilution Parallelism

4 serum samples with different levels of human IFN-α were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 84% and 129% with an overall recovery of 102% (see Table 5).

Table 5

Sample	Dilution	Mean Human IFN-α Concentration (pg/ml)		% Recovery of Exp. Val.
		Expected Value	Observed Value	
1		--	5258	
	1:2	2629	2205	84
	1:4	1315	1167	89
	1:8	657	604	92
2		--	3958	
	1:2	1979	1897	96
	1:4	989	1000	101
	1:8	495	552	112
3		--	3339	
	1:2	1669	1445	87
	1:4	835	861	103
	1:8	417	409	98
4		--	1702	
	1:2	851	949	112
	1:4	426	541	127
	1:8	213	274	129

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human IFN-α levels determined.
There was no significant loss of human IFN-α immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IFN-α level determined after 24 h. There was no significant loss of human IFN-α immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human IFN-α.
To define the specificity of this ELISA several proteins were tested for cross reactivity. Cross reactivity has been shown with natural human Leukocyte IFN (IFN-α), IFN-α2a, IFN-α2b and IFN-α2c.
There was no cross reactivity observed with human IFN-α1, IFN-β (Fibroblast IFN), IFN-γ, IFN-ω, TNF-α, TNF-β, IL-2, IL-6, IL-8 and IL-10.

13.7 Expected Values

Panels of 40 serum as well as plasma (EDTA, citrate, heparin) samples from randomly selected apparently healthy donors (males and females) were tested for human IFN-α.
For detected human IFN-α levels see Table 6:

Table 6

Sample Matrix	Number of Samples Evaluated	Range (pg/ml)	% Detectable	Mean of Detectable (pg/ml)
Serum	40	n.d.	0	--
Plasma (EDTA)	40	n.d. – 76	2.5	76
Plasma (Citrate)	40	n.d.	0	--
Plasma (Heparin)	40	n.d.	0	--

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

14 Ordering Information

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* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

15.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20 x (5 ml) to 95 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 130 µl distilled water to sample wells.
- Add 20 µl sample to designated wells.
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) if available on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 3 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.