Instructions for Use

Interferon-alpha ELISA

Enzyme-linked Immunosorbent Assay for quantitative detection of human IFN alpha.

REF **30150434**

Σ **96**

For research use only.
Not for use in diagnostic procedures.

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1 Intended Use

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

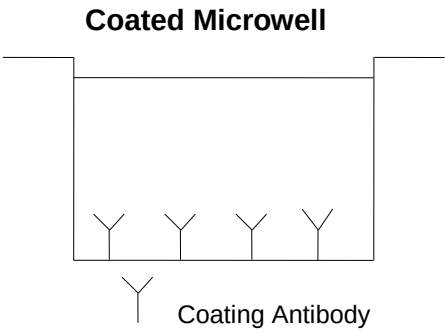
2 Summary

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 alpha), the beta interferon (IFN beta) and the interferon omega (IFN omega) genes (1). In humans the IFN alpha family comprises more than 20 genes and pseudogenes giving rise to 15 different functional gene products. The various species of human IFN alpha are closely related in amino acid sequences with homologies in the range of 80 to 100 %. The molecular weight of the recombinant human IFN alpha species is about 19 kDa consisting of 166 (165 for IFN alpha 2) amino acid residues lacking any N-glycosylation (alpha 14 has N-glycosylation). The cystin mediated disulphide bonds are essential for the biological activity of IFN alpha. The secondary structure of IFN alpha was determined to be mainly alpha-helical. Target analysis of human IFN alpha 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 alpha), 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.
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.

3 Principles of the Test

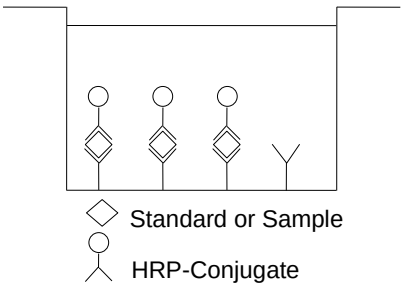
An anti-human IFN alpha coating antibody is adsorbed onto microwells.

Figure 1



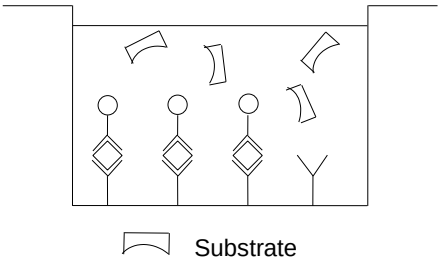
Human IFN alpha present in the sample or standard binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-human IFN alpha antibody is added and binds to human IFN alpha captured by the first antibody.

Figure 2



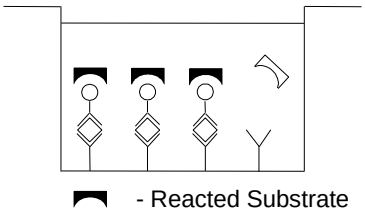
Following incubation unbound HRP-conjugated anti-human IFN alpha is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 3



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

Figure 4



4 Reagents Provided

MTP	1 aluminum pouch with a **Microtiter Plate** (12 strips of 8 wells each) coated with monoclonal antibody to human IFN alpha
ENZCONJ **CONC**	1 vial (200 µL) **HRP-Conjugate** anti-human IFN alpha monoclonal antibody
CAL **LYO**	2 vials human IFN alpha **Standard** lyophilized, 1000 pg/mL upon reconstitution
ASSAYBUF **CONC**	1 vial (5 mL) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20, 10% BSA)
WASHBUF **CONC**	1 bottle (50 mL) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
SUBS	1 vial (15 mL) **Substrate Solution** (tetramethyl-benzidine)
STOP	1 vial (15 mL) **Stop Solution** (1M Phosphoric acid)
	2 Adhesive Films

5 Storage Instructions – ELISA Kit

Store kit reagents between 2° and 8°C.
Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
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, this reagent is not contaminated by the first handling.

6 Specimen Collection and Storage Instructions

Cell culture supernatant, serum and plasma (EDTA, citrate and heparin) were tested with this assay. Other body fluids might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IFN alpha. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).

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
- 50 µL to 300 µL adjustable multichannel micropipette 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 regression analysis

8 Precautions for Use

- All chemicals 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 statement(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.
- 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 the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- 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

1. Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1 Wash Buffer

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

9.2 Assay Buffer (1x)

1. Pour the entire contents (5 mL) 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 to avoid foaming.
2. Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 HRP-Conjugate

Note: The HRP-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **HRP-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

9.4 Human IFN alpha Standard

Reconstitute **human IFN alpha standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 1000.0 pg/mL). Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
The standard has to be used immediately after reconstitution and cannot be stored.

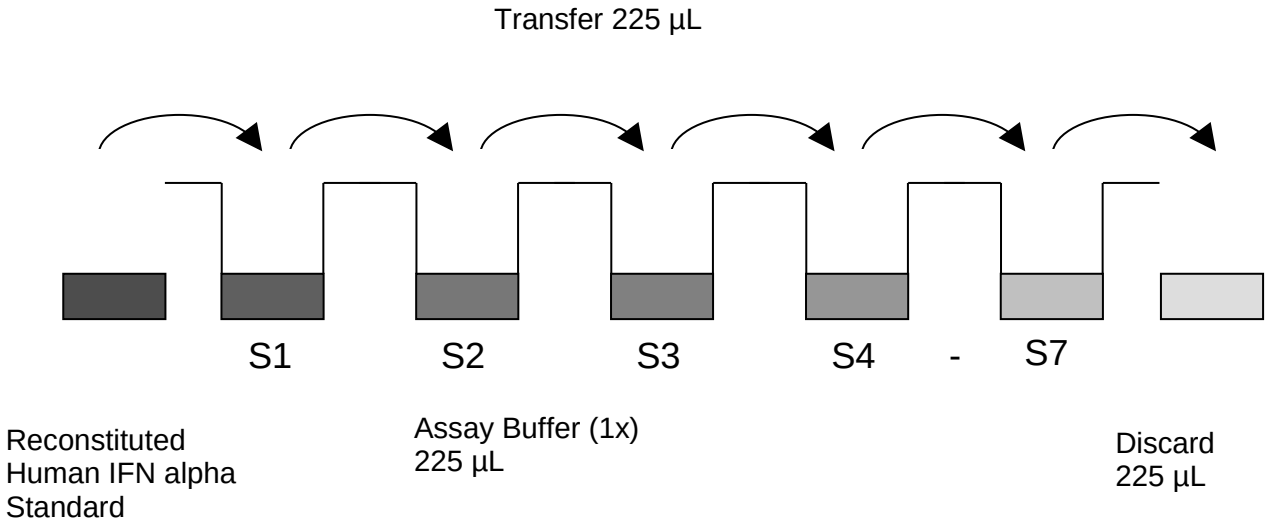
Standard dilutions can be prepared directly on the microtiter plate (see 10.c) or alternatively in tubes (see 9.4.1).

9.4.1 External Standard Dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7
2. Prepare 1:2 serial dilutions for the standard curve as follows:
   Pipette 225 µL of Assay Buffer (1x) into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 1000 pg/mL) into the first tube, labelled S1, and mix (concentration of Standard 1 = 500 pg/mL).
4. Pipette 225 µL of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 5).

   Assay Buffer (1x) serves as blank.

Figure 5



10 Test Protocol

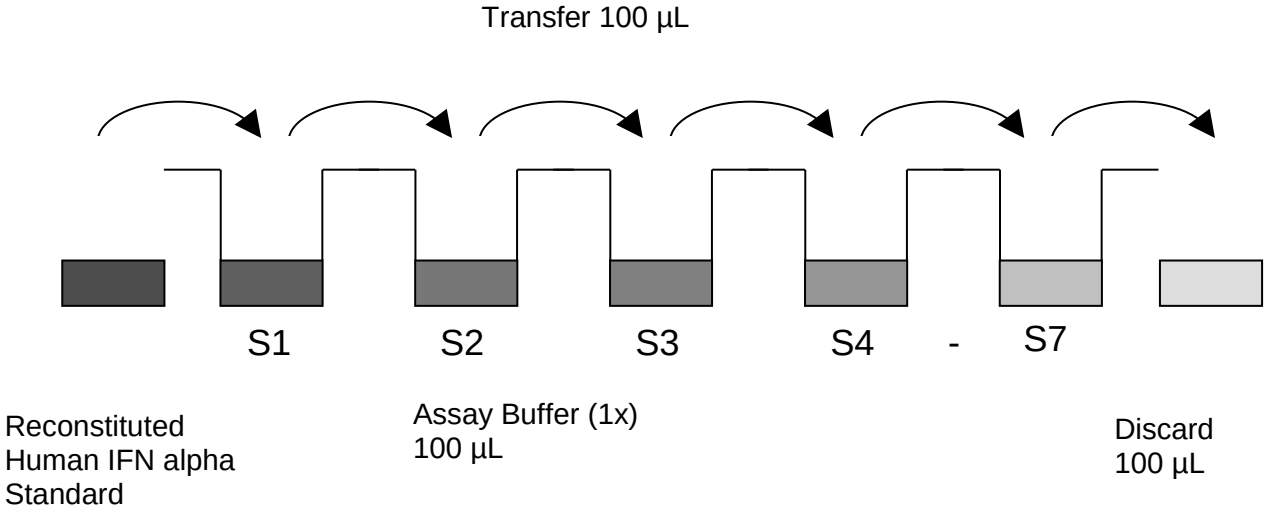
Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

a. 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 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Wash the microwell strips twice 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 step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. **Standard dilution on the microwell plate**
   (Alternatively the standard dilution can be prepared in tubes - see 9.4.1):
   Add 100 µL of Assay Buffer (1x) in duplicate to all **standard wells**. Pipette 100 µL of prepared **standard** (see Preparation of Standard 9.4, concentration = 1000 pg/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1 S1 = 500.0 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IFN alpha standard dilutions, ranging from 500.0 to 7.8 pg/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 6



In case of an **external standard dilution** (see 9.4.1), pipette 100 µL of these standard dilutions (S1 to S7) in the standard wells according to Table 1.

Table 1
Example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (500.0 pg/mL)	Standard 1 (500.0 pg/mL)	Sample 1	Sample 1
B	Standard 2 (250.0 pg/mL)	Standard 2 (250.0 pg/mL)	Sample 2	Sample 2
C	Standard 3 (125.0 pg/mL)	Standard 3 (125.0 pg/mL)	Sample 3	Sample 3
D	Standard 4 (62.5 pg/mL)	Standard 4 (62.5 pg/mL)	Sample 4	Sample 4
E	Standard 5 (31.3 pg/mL)	Standard 5 (31.3 pg/mL)	Sample 5	Sample 5
F	Standard 6 (15.6 pg/mL)	Standard 6 (15.6 pg/mL)	Sample 6	Sample 6
G	Standard 7 (7.8 pg/mL)	Standard 7 (7.8 pg/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 100 µL of **Assay Buffer (1x)** in duplicate to the **blank wells**.

e. Add 80 µL of **Assay Buffer (1x)** to the **sample wells**.

f. Add 20 µL of each **sample** in duplicate to the **sample wells**.

g. Prepare **HRP-Conjugate** (see Preparation of HRP-Conjugate 9.3).

h. Add 50 µL of **HRP-Conjugate** to all wells.

i. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker.

j. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.

k. Pipette 100 µL of **TMB Substrate Solution** to all wells.

l. 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.

m. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well. 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.

n. 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 standards.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 % of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IFN alpha 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 alpha 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 alpha concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:5 (20 µL sample + 80 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 5).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IFN alpha levels. Such samples require further external predilution according to expected human IFN alpha values with Assay Buffer (1x) in order to precisely quantitate the actual human IFN alpha level.
- It is suggested that each testing facility establishes a control sample of known human IFN alpha 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 7.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 7
Representative standard curve for human IFN alpha ELISA. Human IFN alpha was diluted in serial 2-fold steps in Assay Buffer (1x).

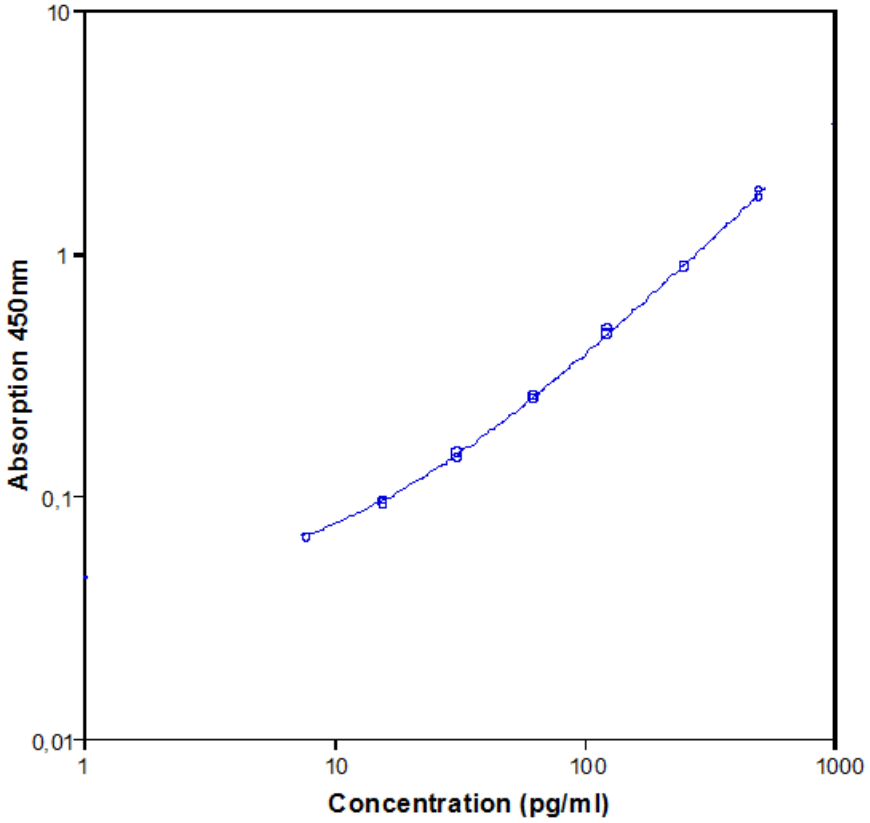


Table 2
Typical data using the human IFN alpha ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human IFN alpha Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	500.0	1.701 1.790	1.746	3.0
2	250.0	0.881 0.876	0.879	0.4
3	125.0	0.462 0.495	0.479	4.9
4	62.5	0.252 0.258	0.255	1.7
5	31.3	0.144 0.149	0.147	2.4
6	15.6	0.093 0.096	0.095	2.2
7	7.8	0.067 0.067	0.067	4.4
Blank	0	0.031 0.028	0.030	7.2

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 false 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 people with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to false 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 alpha 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 3.2 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 8 serum samples containing different concentrations of human IFN alpha. 2 standard curves were run on each plate. Data below show the mean human IFN alpha concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 4.0%.

Table 3
The mean human IFN alpha concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human IFN alpha Concentration (pg/mL)	Coefficient of Variation (%)
1	1	2299	2.2
	2	2343	6.6
	3	2333	1.6
2	1	2192	1.7
	2	2265	2.2
	3	2470	4.6
3	1	1576	4.6
	2	1645	2.0
	3	1429	1.4
4	1	324	4.3
	2	317	2.2
	3	317	4.3
5	1	192	3.7
	2	250	3.3
	3	283	1.5
6	1	538	6.2
	2	541	8.6
	3	425	1.8
7	1	135	9.7
	2	146	8.3
	3	140	7.9
8	1	439	2.1
	2	403	3.0
	3	440	1.1

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 8 serum samples containing different concentrations of human IFN alpha. 2 standard curves were run on each plate. Data below show the mean human IFN alpha 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 7.2%.

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

Sample	Mean Human IFN alpha Concentration (pg/mL)	Coefficient of Variation (%)
1	2345	2.0
2	2309	6.2
3	1550	7.1
4	319	1.2
5	242	19.1
6	501	13.1
7	141	3.9
8	428	4.9

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IFN alpha into serum. Recoveries were determined in 3 independent experiments with 6 replicates each.
The unspiked serum was used as blank in these experiments.
The recovery ranged from 85% to 98% with an overall mean recovery of 92%.

13.4 Dilution Parallelism

Serum samples with different levels of human IFN alpha were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 88% to 123% with an overall recovery of 108% (see Table 5).

Table 5

Sample	Dilution	Expected Human IFN alpha Concentration (pg/mL)	Observed Human IFN alpha Concentration (pg/mL)	Recovery of Expected Concentration (%)
1	1:5	--	812	--
	1:10	406	459	113
	1:20	203	225	111
	1:40	101	125	123
2	1:5	--	2113	--
	1:10	1056	1280	121
	1:20	528	599	113
	1:40	264	322	122
3	1:5	--	2644	--
	1:10	1322	1203	91
	1:20	661	596	90
	1:40	330	289	88
4	1:5	--	2248	--
	1:10	1124	1237	110
	1:20	562	603	107
	1:40	281	301	107

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human IFN alpha levels determined. There was no significant loss of human IFN alpha 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 and at 37°C, and the human IFN alpha level determined after 24 h. There was no significant loss of human IFN alpha immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human IFN alpha.
The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IFN alpha positive serum.
Cross reactivity has been shown with natural human Leukocyte IFN-IFN alpha, IFN alpha 2a, IFN alpha 2b and IFN alpha 2c. No cross-reactivity was observed with human IFN alpha 1, IFN beta (Fibroblast IFN), IFN gamma, IFN omega, TNF alpha, TNF beta, IL-2, IL-6, IL-8 and IL-10.

14 Reagent Preparation Summary

14.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50 mL) to 950 mL distilled water.

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

14.2 Assay Buffer (1x)

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

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

14.3 HRP-Conjugate

Make a 1:100 dilution of **HRP-Conjugate** in Assay Buffer (1x).

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

14.4 Human IFN alpha Standard

Reconstitute lyophilized **human IFN alpha standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

15 Test Protocol Summary

Note: If instructions in this protocol have been followed, samples have been diluted 1:5 (20 µL sample + 80 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 5).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µL Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100 µL reconstituted standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells. Alternatively external standard dilution in tubes (see 9.4.1): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Assay Buffer (1x), in duplicate, to the blank wells.
5. Add 80 µL Assay Buffer (1x) to sample wells.
6. Add 20 µL sample in duplicate, to designated sample wells.
7. Prepare HRP-Conjugate.
8. Add 50 µL HRP-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Empty and wash microwell strips 3 times with Wash Buffer.
11. Add 100 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18°to 25°C).
13. Add 100 µL Stop Solution to all wells.
14. Blank microwell reader and measure colour intensity at 450 nm.

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.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