

# Human IFN $\alpha$ ELISA Kit

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

**Catalog Numbers** BMS216 and BMS216TEN

**Pub. No.** MAN0016566 **Rev.** A.0 (30)

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

The Human IFN $\alpha$  ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IFN  $\alpha$ .

## 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. 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.

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 IFN  $\alpha$  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 our website.

## Principles of the test

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

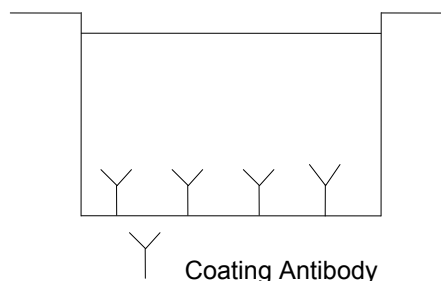


Fig. 1 Coated microwell

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.

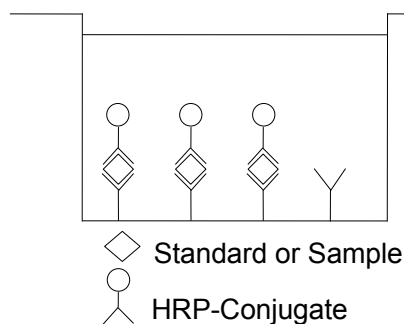


Fig. 2 First incubation

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.

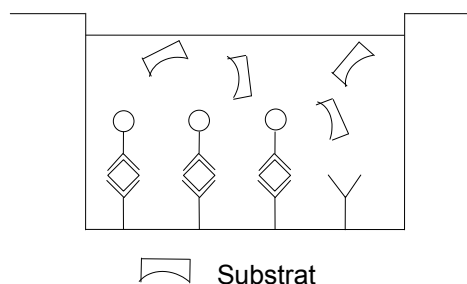


Fig. 3 Second incubation

A colored 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.

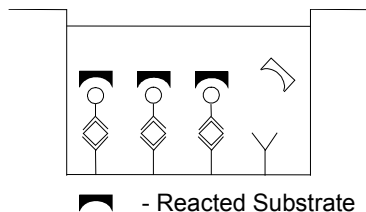


Fig. 4 Stop reaction

## Reagents provided

### Reagents for human IFN $\alpha$ ELISA BMS216 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human IFN  $\alpha$

1 vial (200  $\mu$ L) HRP-Conjugate anti-human IFN  $\alpha$  monoclonal antibody

2 vials human IFN  $\alpha$  Standard lyophilized, 1000 pg/mL upon reconstitution

1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

2 Adhesive Films

### Reagents for human IFN $\alpha$ ELISA BMS216TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human IFN  $\alpha$

10 vials (200  $\mu$ L) HRP-Conjugate anti-human IFN  $\alpha$  monoclonal antibody

10 vials human IFN  $\alpha$  Standard lyophilized, 1000 pg/mL upon reconstitution

2 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

3 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

10 Adhesive Films

## Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C.

Immediately after use remaining reagents should be returned to cold storage (2°C 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.

## Sample 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 samples.

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°C to 8°C (for sample stability refer to "Sample stability" on page 6).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

## Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
- 50  $\mu$ L to 300  $\mu$ L 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

## 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 pipet 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- 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 samples that 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 samples and all potentially contaminated materials as if 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.

## 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.

## 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°C 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

## 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°C 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

## 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

## 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.

## 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  $\mu$ L of Assay Buffer (1x) into each tube.
3. Pipette 225  $\mu$ L of reconstituted standard (concentration = 1000 pg/mL) into the first tube, labeled S1, and mix (concentration of Standard 1 = 500 pg/mL).
4. Pipette 225  $\mu$ L of this dilution into the second tube, labeled 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.

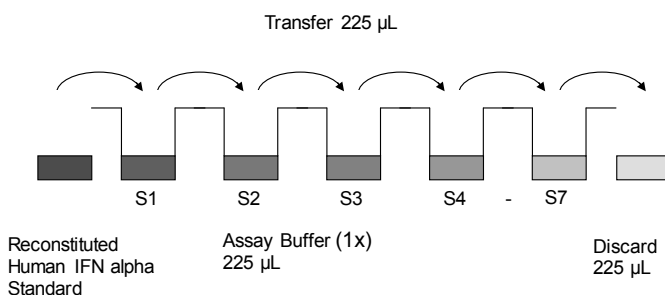


Fig. 5 Dilute standards - tubes

## 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.

1. 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°C to 8°C sealed tightly.
2. Wash the microwell strips twice with approximately 400  $\mu$ L 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.
3. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see “External standard dilution” on page 3):

Add 100  $\mu$ L of Assay Buffer (1x) in duplicate to all standard wells. Pipette 100  $\mu$ L of prepared standard (see “Human IFN  $\alpha$  standard” on page 3, 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  $\mu$ L 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  $\mu$ L of the contents from the last microwells (G1, G2) used.

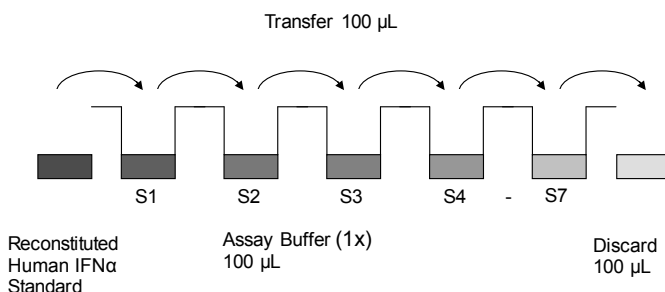


Fig. 6 Dilute standards - microwell plate.

**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

In case of an external standard dilution (see “External standard dilution” on page 3), pipette 100 µL of these standard dilutions (S1–S7) in the standard wells according to Table 1.

- Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.
- Add 80 µL of Assay Buffer (1x) to the sample wells.
- Add 20 µL of each sample in duplicate to the sample wells.
- Prepare HRP-Conjugate (see “HRP-Conjugate” on page 3).
- Add 50 µL of HRP-Conjugate to all wells.
- Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours on a microplate shaker.
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to Step 2 of the test protocol. Proceed immediately to the next step.
- Pipette 100 µL of TMB Substrate Solution to all wells.
- Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

The color 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 color development has to be done individually for each assay.

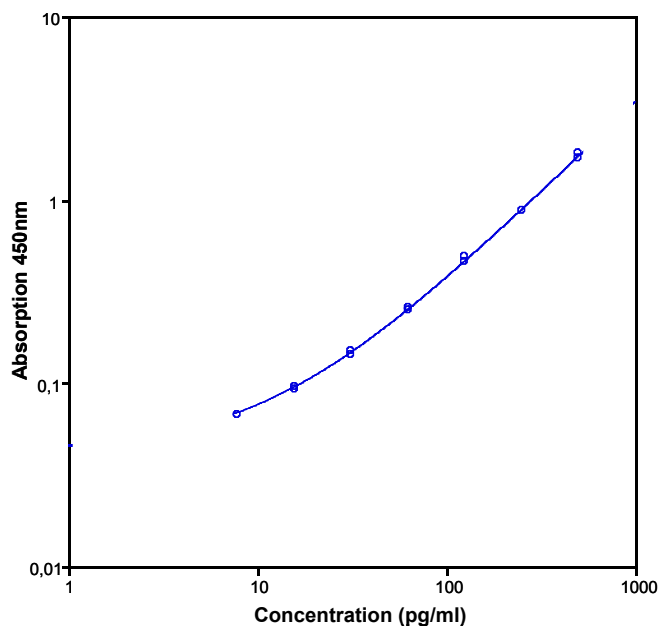
It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.

- 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°C to 8°C in the dark.
- 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.

## 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 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.
- 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 (× 5).
- 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 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.



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

**Table 2** Typical data using the human IFN α 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	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 color intensity. Values measured are still valid.

## 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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

## Performance characteristics

### 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).

### Reproducibility

#### 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  $\alpha$ . Two 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

### 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  $\alpha$ . Two 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

### 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 85–98% with an overall mean recovery of 92%.

## Dilution parallelism

Serum samples with different levels of human IFN  $\alpha$  were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 88% to 123% with an overall recovery of 108%.

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	1,237	110
	1:20	562	603	107
	1:40	281	301	107

## Sample stability

### Freeze-Thaw stability

Aliquots of serum samples (unspiked or spiked) were stored at  $-20^{\circ}\text{C}$  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.

### Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at  $-20^{\circ}\text{C}$ ,  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ , room temperature, and at  $37^{\circ}\text{C}$ , and the human IFN  $\alpha$  level determined after 24 hours. There was no significant loss of human IFN  $\alpha$  immunoreactivity detected during storage under above conditions.

## 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.

## Expected values

Panels of 40 serum as well as EDTA, citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for human IFN  $\alpha$ . Elevated human IFN  $\alpha$  levels depend on the type of immunological disorder. The levels measured may vary with the sample collection used.

Sample matrix	Number of samples evaluated	Range (pg/mL)	% detectable	Mean of detectable (pg/mL)
Serum	40	nd <sup>[1]</sup>	0	–
Plasma (EDTA)	40	nd <sup>[1]</sup> –75.7	2.5	–
Plasma (citrate)	40	nd <sup>[1]</sup>	0	–
Plasma (heparin)	40	nd <sup>[1]</sup> – 54.5	2.5	–

<sup>[1]</sup> nd = nondetectable, samples measured below the lowest standard point are considered to be nondetectable.

## Reagent preparation summary

### 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

### 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

### 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

### Human IFN $\alpha$ standard

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

## Test protocol summary

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

- Determine the number of microwell strips required.
- Wash microwell strips twice with Wash Buffer.
- Standard dilution on the microwell plate: Add 100  $\mu\text{L}$  Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100  $\mu\text{L}$  reconstituted standard into the first wells and create standard dilutions by transferring 100  $\mu\text{L}$  from well to well. Discard 100  $\mu\text{L}$  from the last wells.  
Alternatively, external standard dilution in tubes (see “External standard dilution” on page 3): Pipette 100  $\mu\text{L}$  of these standard dilutions in the microwell strips.
- Add 100  $\mu\text{L}$  Assay Buffer (1x), in duplicate, to the blank wells.
- Add 80  $\mu\text{L}$  Assay Buffer (1x) to sample wells.
- Add 20  $\mu\text{L}$  sample in duplicate, to designated sample wells.
- Prepare HRP-Conjugate.
- Add 50  $\mu\text{L}$  HRP-Conjugate to all wells.
- Cover microwell strips and incubate 2 hours at room temperature ( $18^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ).
- Empty and wash microwell strips 3 times with Wash Buffer.
- Add 100  $\mu\text{L}$  of TMB Substrate Solution to all wells.
- Incubate the microwell strips for about 10 minutes at room temperature ( $18^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ).
- Add 100  $\mu\text{L}$  Stop Solution to all wells.
- Blank microwell reader and measure color intensity at 450 nm.

## Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support

- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

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