

## Human IFN $\gamma$ ELISA KIT

Catalog No. CDK023B

Quantity: 2 x 96 tests

### PRODUCT SPECIFICATIONS :

**Specificity :** Recognizes both natural and recombinant human IFN $\gamma$

**Range :** 12.5 pg / ml - 400 pg / ml

**Sensitivity :** < 5 pg/ml

**Incubation :** 2 h 40 min

**Sample Types :** Serum  
Plasma  
Cell culture supernatant

**Cross Reaction :** No cross reactivity with other human cytokines  
Cross reactivity with simian IFN- $\gamma$

**Kit Content :** Pre-coated 12 strip plate, biotinylated secondary antibody, standards, controls, buffers, Streptavidin-HRP, TMB Substrate, Stop Reagent.

### 1. INTENDED USE

The Cell Sciences IFN $\gamma$  ELISA kit is a solid phase sandwich ELISA for the *in vitro* qualitative and quantitative determination of IFN $\gamma$  in supernatants, buffered solutions or serum and plasma samples and other body fluids. This assay will recognise both natural and recombinant human IFN $\gamma$ .

**This kit has been configured for research use only and must not be used for diagnostic purposes.**

### 2. PRINCIPLE OF THE METHOD

A capture Antibody highly specific for IFN $\gamma$  has been coated to the wells of the microtitre strip plate provided during manufacture. Binding of IFN $\gamma$  samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-IFN $\gamma$  secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of IFN $\gamma$  present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IFN $\gamma$  in any sample tested.



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### 3. REAGENTS PROVIDED AND RECONSTITUTION

Reagents (Store @ 2-8°C)	Quantity 2 x 96 well kit	Reconstitution
P: 96 well microtiter strip plate	2	Ready to use (Pre-coated).
A: Plastic plate covers	4	n/a
B: Standard: 400 pg/ml	4	Reconstitute as directed on the vial (see Assay preparation, section 8).
C: Standard Diluent (Buffer)	1 (25 ml)	10x Concentrate, dilute in distilled water (see Assay preparation, section 8).
D: Standard Diluent (Human Serum)	2 (7.0 ml)	Ready to use.
E: Control	4	Reconstitute as directed on the vial (see Assay preparation, section 8).
F: Biotinylated anti-IFN $\gamma$	2 (0.4 ml)	Dilute in Biotinylated Antibody Diluent (see Assay preparation, section 8).
G: Biotinylated Antibody Diluent	1 (13 ml)	Ready to use.
H: Streptavidin-HRP	4 (5 $\mu$ l)	Add 0.5 ml of HRP Diluent prior to use (see Assay preparation, section 8).
I: HRP Diluent	1 (23 ml)	Ready to use.
J: Wash Buffer	2 (10 ml)	200x Concentrate dilute in distilled water (see Assay preparation, section 8).
K: TMB Substrate	1 (24 ml)	Ready to use.
L: H $_2$ SO $_4$ stop reagent	2 (11 ml)	Ready to use.

### 4. MATERIAL REQUIRED BUT NOT PROVIDED

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000  $\mu$ l adjustable single channel micropipettes with disposable tips
- 50-300  $\mu$ l multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

### 5. STORAGE INSTRUCTIONS

Store kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C). The expiration date of the kit and reagents is stated on box front labels. The expiration 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.



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## 6. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, serum, plasma or other biological samples are suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

**Cell culture supernatants:** Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

**Serum:** Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following that, after clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

**Plasma:** EDTA, citrate, or heparin collected plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

**Storage:** If not analyzed shortly after collection, samples should be aliquoted (250-500  $\mu$ l) to avoid repeated freeze-thaw cycles and stored frozen at -80°C. Avoid multiple freeze-thaw cycles of frozen specimens.

**Recommendation:** Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that the sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

## 7. SAFETY AND PRECAUTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual: "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- The human serum included in this kit have been tested and found non-reactive for HBsAg, anti HIV1 & 2 and anti-HCV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H<sub>2</sub>SO<sub>4</sub> and TMB substrate. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination. For the dispensing of H<sub>2</sub>SO<sub>4</sub> and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.



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- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. **Warning TMB is toxic avoid direct contact with hands. Dispose of properly.**
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances immediately after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution immediately after washing microtiter plate.

## 8. ASSAY PREPARATION

Bring all reagents to room temperature before use.

### 8.1 Assay Design

Determine the number of microwell strips required to test the desired number of samples plus the appropriate number of wells needed for running zeros and standards. Each sample, standard, and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

**Example plate layout** (example shown for a 6 point standard curve)

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	400	400										
B	200	200										
C	100	100										
D	50	50										
E	25	25										
F	12.5	12.5										
G	zero	zero										
H	control	control										

*All remaining empty wells can be used to test samples in duplicate.*

### 8.2 Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2°-8°C.



## 8.3 Preparation of Standard Diluent Buffer

Add the contents of the vial (10x concentrate) to 225 ml of distilled water before use. This solution can be stored at 2-8°C for up to 1 week.

## 8.4 Preparation of Standard

Depending on the type of samples you are assaying, the kit includes two standard diluents because biological fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. You should reconstitute standard vials with the most appropriate standard diluent.

For **serum and plasma** samples: use Standard Diluent for human serum.

For **cell culture supernatants**: use Standard Diluent Buffer.

Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 400 pg/ml of IFN $\gamma$ . Mix the reconstituted standard gently by repeated aspiration/ejection. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 400 to 12.5 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution, add 200  $\mu$ l of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 400 pg/ml.
- Add 100  $\mu$ l of appropriate Standard Diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100  $\mu$ l from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100  $\mu$ l from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 400 pg/ml to 12.5 pg/ml.
- Discard 100  $\mu$ l from the final wells of the standard curve (F1 and F2).

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

## 8.5. Preparation of Control

Freeze-dried control vials should also be reconstituted with the most appropriate standard diluent to your samples.

For **serum and plasma** samples: use Standard diluent - human serum

For **cells culture supernatants**: use Standard diluent buffer.

The Control must be reconstituted with the volume of standard buffer diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume will give a solution for which the concentration is stated on the vial. Do not store after use.



## 8.6. Preparation of Biotinylated anti-IFN $\gamma$

It is recommended that this reagent is prepared immediately before use. Dilute the biotinylated anti-IFN $\gamma$  with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of wells required	Biotinylated Antibody ( $\mu$ l)	Biotinylated Antibody Diluent ( $\mu$ l)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

## 8.7. Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5  $\mu$ l vial with 0.5 ml of HRP diluent **immediately before use**. Do not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of wells required	Streptavidin-HRP ( $\mu$ l)	Streptavidin-HRP Diluent ( $\mu$ l)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10



## 9. METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.

Prepare all reagents as shown in section 8.

**Note:** Final preparation of Biotinylated anti-IFN $\gamma$  (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use

Assay Step		Details
1.	Addition	<b>Prepare Standard curve</b> as shown in section 8.4 above
2.	Addition	Add 100 $\mu$ l of each <b>standard, sample, control, and zero</b> (appropriate standard diluent) in duplicate to the appropriate number of wells.
3.	Addition	Add 50 $\mu$ l of diluted <b>biotinylated anti-IFN<math>\gamma</math></b> to all wells.
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for <b>2 hours</b> .
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.3 ml of <b>1x washing solution</b> into each well. c) Aspirate the contents of each well. d) Repeat step b and c another two times.
6.	Addition	Add 100 $\mu$ l of <b>Streptavidin-HRP</b> solution into all wells.
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for <b>30 min</b> .
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100 $\mu$ l of ready-to-use <b>TMB Substrate Solution</b> into all wells.
10.	Incubation	Incubate in the dark for <b>15-20 minutes*</b> at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100 $\mu$ l of <b>H<sub>2</sub>SO<sub>4</sub> Stop Reagent</b> into all wells.
<p><b>Read the absorbance</b> value of each well (immediately after step 10) on a spectrophotometer using 450 nm as the primary wave length and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).</p>		

*\*Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*



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## 10. DATA ANALYSIS

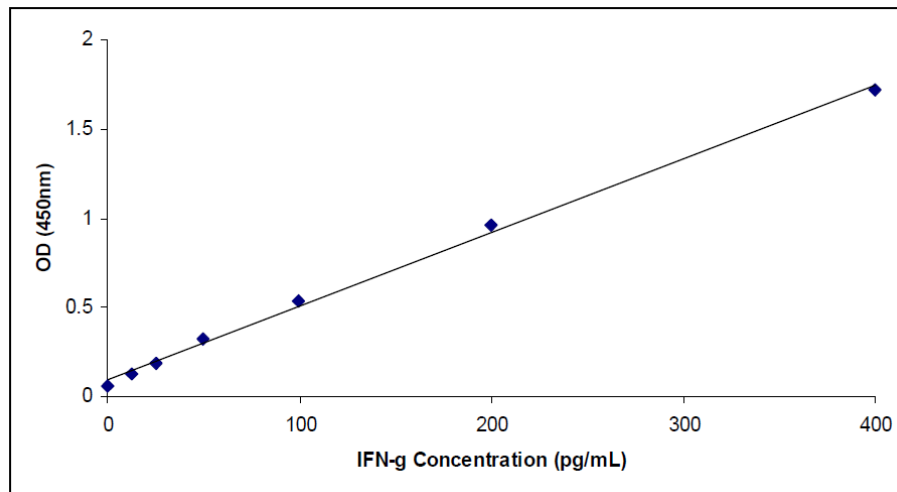
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IFN $\gamma$  standard concentration on the horizontal axis.

The amount of IFN $\gamma$  in each sample is determined by extrapolating OD values against IFN $\gamma$  standard concentrations using the standard curve.

### Example IFN $\gamma$ Standard curve

Standard	IFN $\gamma$ Conc	OD (450nm) mean	CV (%)
1	400	1.72	1.0
2	200	0.96	7.0
3	100	0.54	1.8
4	50	0.32	3.3
5	25	0.19	1.9
6	12.5	0.13	0.0
zero	0	0.06	-



**Note:** Curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.



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## 11. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples, always multiply results by the appropriate dilution factor to produce the actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer. Fill wells with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred. Reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays, conditions may vary from assay to assay. Therefore, **a fresh standard curve must be prepared and run for every assay.**

## 12. PERFORMANCE CHARACTERISTICS

### 12.1 Sensitivity

The minimum detectable dose of IFN $\gamma$  was determined to be less than **5 pg/ml**. This was determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 40 times.

### 12.2 Specificity

The assay recognizes both natural and recombinant human IFN $\gamma$ . To define the specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (IL-1 $\alpha$ , IL-1 $\beta$ , IL-10, IL-12, IL-4, IL-6, TNF $\alpha$ , IL-8, and IL-13).



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## 12.3 Precision

### Intra-assay

Reproducibility within the assay was evaluated in three independent experiments by two different analysts. Each assay was carried out with 6 replicates (3 duplicates) of 3 spiked human pooled serum samples and 2 supernatants containing different concentrations of IFN $\gamma$ . Data below shows the mean IFN $\gamma$  concentration and the coefficient of variation for each sample. **The overall intra-assay coefficient of variation was calculated to be 4.9%**

Session	Samples	Assay 1 IFN $\gamma$ pg/ml	Assay 2 IFN $\gamma$ pg/ml	Assay 3 IFN $\gamma$ pg/ml	Mean	SD	CV (%)
1	1	282	267	271	273	7.8	2.9
	2	144	151	149	148	3.5	2.3
	3	62	68	69	66	3.6	5.5
	4	68	62	55	62	6.9	11.1
	5	177	180	185	181	4.4	2.5
2	1	275	263	278	272	7.7	2.8
	2	150	146	150	149	2.1	1.4
	3	56	63	69	63	6.6	10.6
	4	83	77	71	77	5.9	7.7
	5	196	185	166	183	15.3	8.4
3	1	262	242	257	254	10.1	4
	2	127	132	130	130	2.3	1.8
	3	53	48	56	52	3.8	7.2
	4	65	63	66	65	1.7	2.6
	5	165	164	160	163	2.9	1.8

**Note:** Example data shown above is the data generated by analyst A only.



## Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two analysts. Each assay was carried out with 6 replicates of 3 spiked serum human pooled serum samples and 2 supernatants containing different concentrations of IFN $\gamma$ . **The calculated overall coefficient of variation was 10%**

Technician	Session	Sample 1 IFN $\gamma$ pg/ml	Sample 2 IFN $\gamma$ pg/ml	Sample 3 IFN $\gamma$ pg/ml	Sample 4 IFN $\gamma$ pg/ml	Sample 5 IFN $\gamma$ pg/ml
A	1	282	144	62	68	177
		267	151	68	62	180
		271	149	69	55	185
	2	275	150	56	83	196
		263	146	63	77	185
		278	150	69	71	166
	3	262	127	53	65	165
		242	132	48	63	164
		257	130	56	66	160
B	1	270	142	44	82	191
		251	139	46	76	182
		261	136	51	73	180
	2	266	115	54	92	207
		245	125	62	94	208
		228	117	48	86	201
	3	283	146	55	77	162
		259	139	60	73	162
		289	145	52	79	163
Mean		264	138	56	75	180
SD		16	11	8	11	16
CV		6	8	14	14	9

## 12.4 Dilution Parallelism

Three serum samples and one human pooled serum (spiked) with different levels of IFN $\gamma$  were analyzed at different serial two fold dilutions (1:2 to 1:8) with two replicates each. Recoveries ranged from 87 to 126% with an overall **mean recovery of 107%**.



## 12.5 Spike Recovery

The spike recovery was evaluated by spiking 3 concentrations of IFN $\gamma$  in human serum in 2 separate experiments. Recoveries ranged from 81 to 100% with an overall **mean recovery of 94%**.

## 12.6. Stability

### Storage Stability

Aliquots of spiked serum samples were stored at  $-20^{\circ}\text{C}$ ,  $2-8^{\circ}\text{C}$ , room temperature (RT) and at  $37^{\circ}\text{C}$  and the IFN $\gamma$  level determined after 24 h. There was no significant loss of IFN $\gamma$  reactivity during storage at RT and  $2-8^{\circ}\text{C}$  and a slight loss after 24 h at  $37^{\circ}\text{C}$ .

### Freeze-thaw Stability

Aliquots of spiked serum were stored frozen at  $-20^{\circ}\text{C}$  and thawed up to 5 times and the IFN $\gamma$  level was determined. There was a slight loss (11%) of IFN $\gamma$  after 5 cycles of freezing and thawing.

## 12.7. Expected serum values

A panel of 40 human sera and 40 human plasma was tested for IFN $\gamma$ . All were below 5 pg/ml except one sample with a concentration of 7.5 pg/ml for serum and 24 pg/ml for plasma.

## 12.8. Standard Calibration

This immunoassay is calibrated against the International Reference Standard NIBSC 88/606. NIBSC 88/606 is quantitated in International Units (IU), 1IU corresponding to 0.28ng calibration IFN $\gamma$ .



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## 13. ASSAY SUMMARY

Total procedure length : 2 h 45 min

Add 100 µl of sample and diluted standard/controls and 50 µl Biotinylated anti-IFN $\gamma$



Incubate 2 hours at room temperature



Wash three times



Add 100 µl of Streptavidin-HRP



Incubate 30 min at room temperature



Wash three times



Add 100 µl of ready-to-use TMB  
Protect from light. Let the color develop for 15-20 min.



Add 100µl H<sub>2</sub>SO<sub>4</sub>



Read Absorbance at 450 nm

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE**



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