

## Mouse IFN-gamma ELISA KIT

**Catalog No.** 860.050.048  
860.050.096  
860.050.192

**Quantity:** 1 x 48 Wells  
1 x 96 Wells  
2 x 96 Wells

### INTENDED USE

The Mouse IFN $\gamma$  ELISA is to be used for the in-vitro quantitative determination of mouse interferon- $\gamma$  (mIFN $\gamma$ ) in mouse serum, buffered solutions or cell culture medium. The assay will recognize both natural and recombinant mouse IFN $\gamma$ .

### PRINCIPLE OF THE METHOD

The mouse IFN $\gamma$  Kit (mIFN $\gamma$ ) is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for mIFN $\gamma$  has been coated onto the wells of the microtiter strips provided. The antigen and a biotinylated polyclonal antibody specific for mIFN $\gamma$  are simultaneously incubated. Revelation step includes Streptavidin-Horse Radish peroxidase and TMB as chromogen.

### REAGENTS PROVIDED AND RECONSTITUTION (Below is for a two plate kit)

| REAGENTS (Store at 2-8°C)                     | 2 x 96 Wells   | 1 x 96 Wells    | COLOR CODE | RECONSTITUTION   |
|---|----------------|-----------------|------------|--|
| 96-wells microtiter plates                    | 2              | 1               |            | Ready-to-use   |
| Plastic cover                                 | 4              | 2               |            |  |
| Standard : 1000 pg/ml                         | 4 vials        | 2 vials         | Yellow     | Reconstitute with the volume of standard diluent indicated on the vial. (See Reagents Preparation on page 2) |
| Standard Buffer Diluent                       | 1 Vial         | 1 vial          | Black      | (25 ml) 10X concentrate<br>Dilute in distilled Water   |
| Biotinylated anti-mIFN $\gamma$               | 2 Vials        | 1 vial          | Red        | (0.4 ml) Dilute in biotinylated antibody diluent   |
| Biotinylated Antibody Diluent                 | 1 Vial (13 ml) | 1 vial (7.5 ml) | Red        | Ready-to-use   |
| Streptavidin-HRP                              | 4 Vials        | 2 vials         |            | (5 $\mu$ l) 0.5 ml of HRP-Diluent before further dilutions   |
| HRP Diluent                                   | 1 Vial         | 1 vial          | Red        | (23 ml) Ready-to-use   |
| Washing Buffer                                | 2 Vials        | 1 vial          | White      | (10 ml) 200X concentrate.<br>Dilute in distilled Water   |
| Chromogen TMB :                               | 1 Vial (24 ml) | 1 vial (11 ml)  |            | Ready-to-use   |
| H <sub>2</sub> SO <sub>4</sub> : Stop Reagent | 2 Vials        | 1 vial          | Black      | (11 ml) Ready-to-use   |

### MATERIAL REQUIRED BUT NOT PROVIDED

- \* Distilled water.
- \* Pipettes : 10  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l, 200  $\mu$ l and 1000  $\mu$ l.
- \* Vortex mixer and magnetic stirrer.



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**SAFETY**

- \* For research use only.
- \* 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.
- \* Avoid any skin contact with H<sub>2</sub>SO<sub>4</sub> and TMB. In case of contact, wash thoroughly with water.
- \* Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- \* Do not pipette with mouth.

**PROCEDURAL NOTES/LAB. QUALITY CONTROL**

1. 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 right after resuspension and use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different kit lots.
5. Do not use reagents beyond the expiration date of the kit.
6. 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.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. 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.
10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
11. If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
12. Serum samples should be collected in pyrogen/endotoxin-free tubes.
13. Samples should be stored frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen specimens. Thaw completely prior to analysis.
14. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
15. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
16. Respect incubation times described in the assay procedure.
17. Dispense the TMB solution within 15 min. following the washing of the microtiter plate.



## **REAGENTS PREPARATIONS**

### *Standard buffer diluent*

Dilute 5 ml of standard buffer diluent in 50 ml of distilled water or all the content of the vial (25 ml) in 250 ml of distilled water.

### *Standards*

Lyophilised standard vials have to be reconstituted with 1.07 mL of standard buffer diluent to give a concentration of 1000 pg/ml mIFN $\gamma$ .

### *Dilution of biotinylated anti- mIFN $\gamma$*

Dilute the biotinylated anti-m IFN $\gamma$  with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

| <b>Number of Wells used</b> | <b>Biotinylated Antibody (<math>\mu</math>l)</b> | <b>Biotinylated Antibody Diluent (<math>\mu</math>l)</b> |
|-----------------------------|--|--|
| 16                          | 40   | 1060   |
| 24                          | 60   | 1590   |
| 32                          | 80   | 2120   |
| 48                          | 120  | 3180   |
| 96                          | 240  | 6360   |
| 192                         | 480  | 12720  |
|                             |  |  |

### *Dilution of Streptavidin-HRP*

**Add 0.5 ml of HRP diluent to a 5  $\mu$ l vial of Streptavidin-HRP . DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS.** Extemporaneous preparations are recommended. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

| <b>Number of Wells</b> | <b>Streptavidin-HRP(<math>\mu</math>l)</b> | <b>Strep-HRP Diluent (ml)</b> |
|------------------------|--|-------------------------------|
| 16                     | 30   | 2                             |
| 24                     | 45   | 3                             |
| 32                     | 60   | 4                             |
| 48                     | 75   | 5                             |
| 96                     | 150  | 10                            |
| 192                    | 300  | 20                            |
|                        |  |                               |



### Dilution of Washing Buffer

Dilute 2 ml of washing buffer in 400 ml of distilled water or all the content of one vial ( 10 ml ) in 2000 ml of distilled water.

### ASSAY METHOD

- a) Before use, mix all reagents thoroughly without making foam.
- b) 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 samples should be assayed in duplicate. Remove sufficient microwell strips from the pouch.
- c) Add 100  $\mu$ l of appropriate standard diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200  $\mu$ l of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100  $\mu$ l from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of mIFN- $\gamma$  standard dilutions ranging from 1000 to 31.25 pg/ml. Discard 100  $\mu$ l from the content of the last microwells used (F1, F2).
- d) Add 100  $\mu$ l of appropriate standard diluent to the blank wells ( G1-G2 ) and 100  $\mu$ l of sample to sample wells.
- e) Preparation of biotinylated anti- mIFN- $\gamma$ : see reagents preparation.
- f) Add 50  $\mu$ l of diluted biotinylated anti- mIFN- $\gamma$  to all wells.
- g) Cover with a plate cover and incubate for 3 hours at room temperature ( 18°C - 25°C ).
- h) Remove the cover plate and wash the plate as follows:
  - 1) aspirate the liquid from each well ;
  - 2) dispense 0.3 ml of washing solution into each well ;
  - 3) aspirate again the content of each well ;
  - 4) Repeat steps 2) and 3) two times.
- i) Prepare HRP solution just before use: see reagents preparation.
- j) Dispense 100  $\mu$ l of HRP solution into all wells, including the blank wells. Put back the cover plate.
- k) Incubate the microwell strips at room temperature for 20 minutes.
- l) Remove plate cover and empty wells. Wash microwell strips according to point (h). Proceed immediately to the next step.
- m) Pipette 100  $\mu$ l of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
- n) Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable (maximum 20 minutes).
- o) The enzyme-substrate reaction is stopped by quickly pipetting 100  $\mu$ l of 1.8 N sulfuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read immediately after the addition of sulfuric acid, or within one hour, if the microwell strips are stored at 2-8°C in the dark.
- p) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

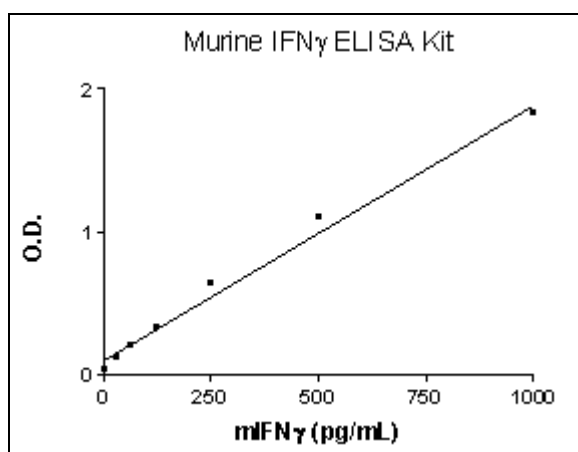


## SUGGESTED PLATE SCHEME

|   | Standard Concentrations<br>pg/mL |       | Sample wells |   |   |   |   |   |   |    |    |    |
|---|----------------------------------|-------|--------------|---|---|---|---|---|---|----|----|----|
|   | 1                                | 2     | 3            | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 1000                             | 1000  |              |   |   |   |   |   |   |    |    |    |
| B | 500                              | 500   |              |   |   |   |   |   |   |    |    |    |
| C | 250                              | 250   |              |   |   |   |   |   |   |    |    |    |
| D | 125                              | 125   |              |   |   |   |   |   |   |    |    |    |
| E | 62.5                             | 62.5  |              |   |   |   |   |   |   |    |    |    |
| F | 31.25                            | 31.25 |              |   |   |   |   |   |   |    |    |    |
| G | Blank                            | Blank |              |   |   |   |   |   |   |    |    |    |
| H |                                  |       |              |   |   |   |   |   |   |    |    |    |

## DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding mIFN $\gamma$  standard concentration on the horizontal axis. The amount of mIFN- $\gamma$  in each sample is determined by extrapolating OD values to mIFN $\gamma$  concentrations using the standard curve.



**Typical mIFN $\gamma$  standard curve ranging from 31.25 to 1000pg/mL**



## **LIMITATIONS OF THE PROCEDURE**

Do not extrapolate the standard curve beyond the 1000 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples ( > 1000 pg/ml ) have to be diluted with standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been thoroughly investigated. The rate of degradation of native mIFN $\gamma$  in various matrices has not been investigated.

## **PERFORMANCES AND CHARACTERISTICS**

### **Sensitivity**

The minimum detectable dose of mIFN $\gamma$  is less than 15 pg/ml.

This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 80 times.

### **Precision**

| Intra-Assay |    |              |      |     | Inter-Assay |    |              |      |     |
|-------------|----|--------------|------|-----|-------------|----|--------------|------|-----|
| Sample      | n  | Mean (pg/mL) | SD   | CV% | Sample      | n  | Mean (pg/mL) | SD   | CV% |
| A           | 20 | 315.4        | 14.5 | 4.6 | A           | 9  | 128.8        | 7.4  | 5.7 |
| B           | 19 | 588.6        | 19.1 | 4.6 | B           | 10 | 546.9        | 25.2 | 4.6 |

### **Linearity of dilution**

A mouse serum pool containing 1000 pg/ml of measured mIFN- $\gamma$  was serially diluted in mouse serum over the range of the assay. Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99.

### **Conversion**

It has been determined that 1 Unit of mouse IFN $\gamma$  corresponds with 50 to 75 pg pure natural and CHO- derived mouse IFN $\gamma$



## ASSAY PROCEDURE SUMMARY

**Total procedure length : 3h45mn**

Add 100µl of sample or diluted standard  
or control



Add 50µl of diluted biotinylated  
detection antibody to all wells



Incubate 3 hours at room temperature



Wash three times



Add 100µl of HRP-Streptavidin to all wells



Incubate 20min at room temperature



Wash three times



Add 100 µl of ready-to-use TMB  
Protect from light. Let the color develop for 12-15 min.



Add 100µl H<sub>2</sub>SO<sub>4</sub>



Read Absorbance at 450nm

