

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse Interferon gamma (IFN- γ) concentrations in serum, plasma and cell culture supernatants.

general information

| | |
|---------------------------|-------------------------|
| Catalogue Number | KE10001 |
| Product Name | IFN- γ ELISA Kit |
| Species cross-reactivity | Mouse IFN- γ |
| Range (calibration Range) | 31.25 - 2000 pg/mL |
| Tested applications | Quantification ELISA |

database links

| | |
|-------------|----------------|
| Entrez Gene | 15978 (Mouse) |
| SwissProt | P01580 (Mouse) |

kit components & storage

| | | |
|---|-----------|-------------------------------|
| Microplate - antibody coated 96-well Microplate (8 well \times 12 strips) | 1 plate | Store at 2-8°C for six months |
| Standard -4000 pg/bottle; lyophilized* | 2 bottles | Store at 2-8°C for six months |
| Detection antibody, biotinylated (100X) - 120 μ L/vial | 1 vial | Store at 2-8°C for six months |
| Streptavidin-HRP (100X) - 120 μ L/vial | 1 vial | Store at 2-8°C for six months |
| Sample Diluent PT 1-ac - 30 mL/bottle. For serum and plasma samples | 1 bottle | Store at 2-8°C for six months |
| Sample Diluent PT 1-ef- 30 mL/bottle. For cell culture supernatants | 1 bottle | Store at 2-8°C for six months |
| Detection Diluent - 30 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| Wash Buffer Concentrate (20X) - 30 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| Stop Solution - 12 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| Plate Cover Seals | 3 pieces | |

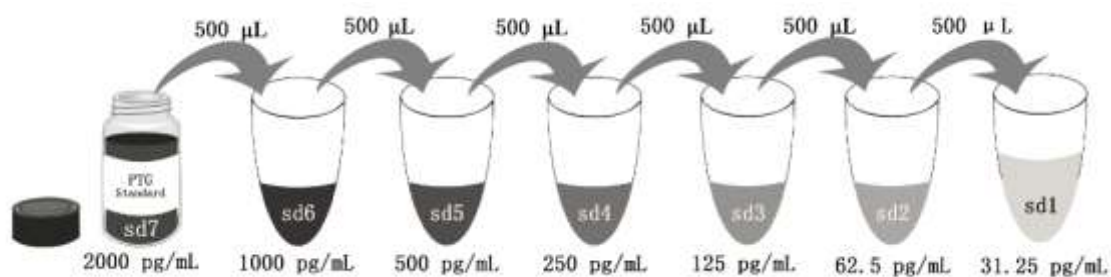
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ac is for standard, serum and plasma.

Sample Diluent PT 1-ef is for standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 1-ac or PT 1-ef in standard. This reconstitution gives a stock solution of 2000 pg/mL.



| | | | | | | | |
|---|---------|--------|--------|--------|--------|--------|--------|
| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| # µL of Sample Diluent PT 1-ac or PT 1-ef | 2000 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

product description

KE10001 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IFN- γ ELISA kit is to be used to detect and quantify protein levels of endogenous IFN- γ . The assay recognizes mouse IFN- γ . An antibody specific for IFN- γ has been pre-coated onto the microwells. The IFN- γ protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IFN- γ is added to detect the captured IFN- γ protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm with the correction wavelength set at 630 nm.

background

The IFNs were originally discovered as agents that interfere with viral replication. Initially, they were classified by the secreting cell type but are now classified into type I and type II according to receptor specificity and sequence homology. Interferon gamma (IFN- γ) is a soluble cytokine that is the only member of the type II class of interferons. It is secreted by Th1 cells, cytotoxic T cells and NK cells. The cytokine is associated with antiviral, immunoregulatory and anti-tumor properties and is a potent activator of macrophages. It plays crucial roles in pathogen clearance. Aberrant Ifng expression is associated with a number of autoinflammatory and autoimmune diseases. It has been identified in many studies as a biomarker for pleural tuberculosis (TB). Mutations in this gene are associated with aplastic anemia.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

safety notes

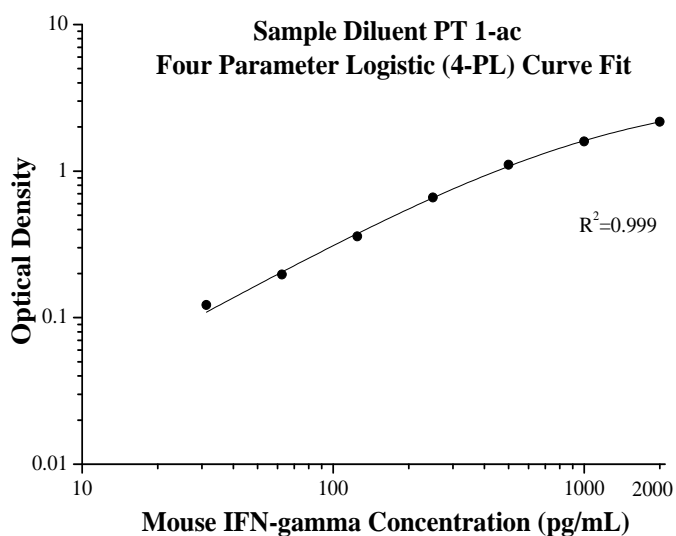
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

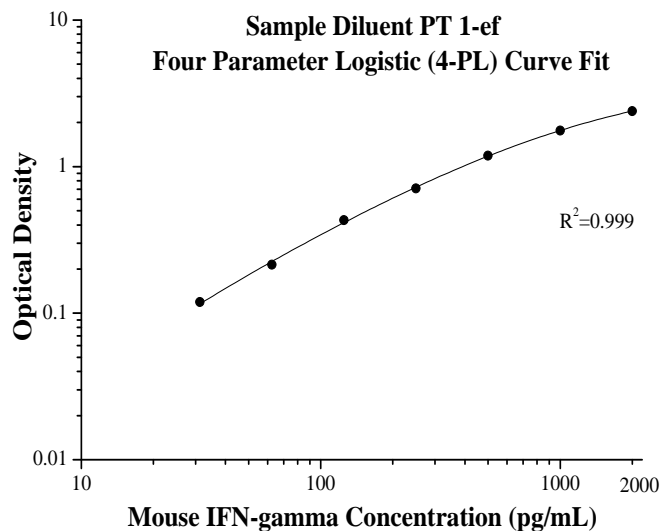
| Step | Reagent | Volume | Incubation | Wash | Notes |
|------|--|--------|----------------|-------------|------------------------------|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



| (pg/mL) | O.D | Average | Corrected |
|---------|-------|---------|-----------|
| 0 | 0.102 | 0.101 | - |
| | 0.101 | | |
| 31.25 | 0.221 | 0.223 | 0.122 |
| | 0.225 | | |
| 62.5 | 0.299 | 0.297 | 0.197 |
| | 0.297 | | |
| 125 | 0.472 | 0.459 | 0.358 |
| | 0.445 | | |
| 250 | 0.745 | 0.761 | 0.660 |
| | 0.777 | | |
| 500 | 1.199 | 1.204 | 1.103 |
| | 1.208 | | |
| 1000 | 1.618 | 1.69 | 1.589 |
| | 1.762 | | |
| 2000 | 2.219 | 2.274 | 2.173 |
| | 2.329 | | |



| (pg/mL) | O.D | Average | Corrected |
|---------|-------|---------|-----------|
| 0 | 0.083 | 0.085 | - |
| | 0.088 | | |
| 31.25 | 0.196 | 0.204 | 0.119 |
| | 0.212 | | |
| 62.5 | 0.292 | 0.299 | 0.214 |
| | 0.305 | | |
| 125 | 0.537 | 0.516 | 0.431 |
| | 0.495 | | |
| 250 | 0.793 | 0.795 | 0.710 |
| | 0.796 | | |
| 500 | 1.288 | 1.271 | 1.186 |
| | 1.253 | | |
| 1000 | 1.856 | 1.847 | 1.762 |
| | 1.838 | | |
| 2000 | 2.439 | 2.479 | 2.394 |
| | 2.519 | | |

precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Sample | Intra-assay Precision | | | Inter-assay Precision | | |
|--------------|-----------------------|-------|---------|-----------------------|-------|-------|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 24 | 24 | 24 |
| Mean (pg/mL) | 207.1 | 367.4 | 1,101.3 | 184.4 | 330.7 | 917.8 |
| SD | 10.3 | 16.5 | 76.1 | 15.0 | 32.4 | 88.9 |
| CV% | 5.0 | 4.5 | 6.9 | 8.2 | 9.8 | 9.7 |

recovery

The recovery of IFN- γ spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated. (The serum sample were initially diluted 1:1)

| Sample Type | | Average% of Expected | Range (%) |
|---------------------------|-----|----------------------|-----------|
| Mouse serum | 1:2 | 89 | 81-94 |
| | 1:4 | 92 | 88-95 |
| Cell culture supernatants | 1:2 | 89 | 79-91 |
| | 1:4 | 95 | 79-104 |

sample values

Mouse lung (1 lung, 1-2 mm pieces) were cultured for 5 days in RPMI1640 plus 15% fetal bovine serum and stimulated with 10 µg/mL PHA. An aliquot of cell culture supernate was removed, assayed for mouse IFN-γ and measured:

| Condition | (pg/mL) |
|-------------------|---------|
| Unstimulated | - |
| Stimulated for 5d | 153 |

sensitivity

The minimum detectable dose of mouse IFN-γ is 5.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of mouse IFN-γ in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The serum sample were initially diluted 1:1)

| | | Mouse serum (Sample Diluent PT 1-ac) | Cell culture supernatants (Sample Diluent PT 1-ef) |
|------|----------------------|---|---|
| 1:2 | Average% of Expected | 97 | 100 |
| | Range (%) | 93-101 | 97-102 |
| 1:4 | Average% of Expected | 96 | 94 |
| | Range (%) | 88-103 | 90-99 |
| 1:8 | Average% of Expected | 98 | 89 |
| | Range (%) | 94-101 | 85-92 |
| 1:16 | Average% of Expected | 96 | 95 |
| | Range (%) | 93-99 | 90-100 |

references

1. Isaacs, A. et al.(1957) The interferon Proc. R. Soc. Lond. B Biol. Sci. 147: 258-267.
2. Gray PW. et al. (1982) Nature. 298 : 859-63.
3. Bullens, D. M. et al.(2001) Int. Immunol. 13: 181-191.
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