

# ELISA for Rat IFN- $\gamma$

Product Code: 3220-1A-20

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CONTENTS, development kit for 20 plates:

**Vial 1 (green)**

Monoclonal antibody rIFN $\gamma$ -I (500  $\mu$ l)

Concentration: 1 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody rIFN $\gamma$ -II (250  $\mu$ l)

Concentration: 1 mg/ml

**Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (250  $\mu$ l)

**Vial 4**

Recombinant rat IFN- $\gamma$  standard (20  $\mu$ g)

To ensure total recovery of stated quantity, vials have been overfilled.

**STORAGE:**

Shipped at ambient temperature. On arrival box 1 should be stored refrigerated at 4-8°C and box 2 should be stored frozen at -20°C.

# General

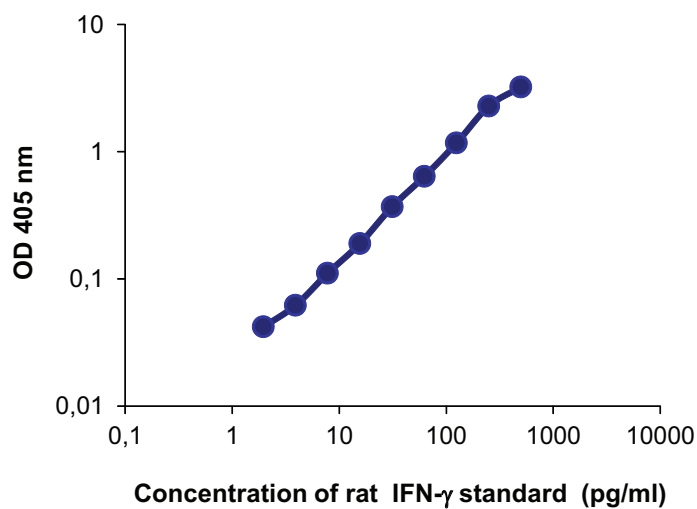
**Intended use:** For quantitative determination of native rat IFN- $\gamma$  in solution, e.g. cell culture supernatant and serum/plasma samples.

**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu\text{m}$ ) PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG..

**Standard range:** 3-300 pg/ml.

**Intra-assay variation:** < 5%

**Standard calibration:** No international standard exists for calibration.



# Guidelines for Rat IFN- $\gamma$ ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb rIFN $\gamma$ -I, diluted to 2  $\mu\text{g}/\text{ml}$  in PBS, pH 7.4, by adding 100  $\mu\text{l}/\text{well}$ . Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200  $\mu\text{l}/\text{well}$ ).
  3. Block plate by adding 200  $\mu\text{l}/\text{well}$  of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.
  4. Wash five times with PBS containing 0.05% Tween.
  5. Prepare rat IFN- $\gamma$  standard by reconstituting contents of vial 4 in 200  $\mu\text{l}$  5 mM Tris pH 8.0+100mM NaCl to a concentration of 0.1 mg/ml. Dilute in PBS with 0.1% BSA to make up a stock solution of 10  $\mu\text{g}/\text{ml}$ . The stock solution should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not be refrozen after initial use. For the test, prepare dilutions of the stock using the standard range as a guideline.
  6. Add 100  $\mu\text{l}/\text{well}$  of samples or standards diluted in incubation buffer and incubate for 2 hours at room temperature.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb rIFN $\gamma$ -II-biotin at 1  $\mu\text{g}/\text{ml}$  in incubation buffer. Incubate for 1 hour at room temperature.
  9. Wash as in step 4.
  10. Add 100  $\mu\text{l}/\text{well}$  of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
  11. Wash as in step 4.
  12. Add 100  $\mu\text{l}/\text{well}$  of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP).
  13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

**NOTE; for research use only.**

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages therefrom.



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2013-01-24

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the following standards:



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