

# Rat IFN- $\gamma$ ELISA development kit

Product Code: 3220-1A-6

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CONTENTS, development kit for 6 plates:

**Vial 1 (green)**

Monoclonal antibody rIFN $\gamma$ -I (150  $\mu$ l)

Concentration: 1 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody rIFN $\gamma$ -II (80  $\mu$ l)

Concentration: 1 mg/ml

**Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (80  $\mu$ 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

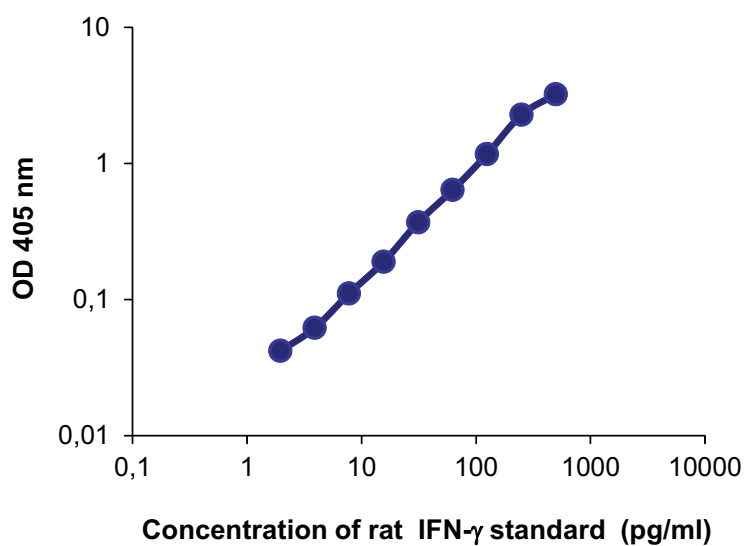
**Intended use:** For quantitative determination of native rat IFN- $\gamma$  in solution, e.g. cell culture supernatant and serum/plasma samples.

**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 0.002% 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), available from Mabtech product code 3652-P10.
  13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



**The products are for research use only.**

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Mabtech AB (Head Office)  
Sweden  
Tel: +46 8 716 27 00  
mabtech@mabtech.com

Mabtech, Inc.  
USA  
Tel: +1 513 871-4500  
mabtech.usa@mabtech.com