# ELISA for Rat IFN-γ

Product Code: 3220-1A-6

# CONTENTS, development kit for 6 plates:

### Vial 1 (green)

Monoclonal antibody rIFNγ-I (150 μl)

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-γ standard (20 μg)

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

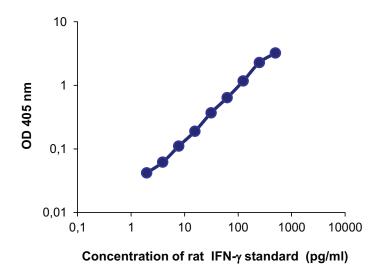
**Intended use:** For quantitative determination of native rat IFN- $\gamma$  in solution, e.g. cell culture supernatant and serum/plasma samples.

**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu$ 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-γ ELISA

- **Day 1** 1. Coat a high protein binding ELISA plate with mAb rIFN $\gamma$ -I, diluted to 2 μg/ml in PBS, pH 7.4, by adding 100 μl/well. Incubate overnight at 4-8°C.
- **Day 2** 2. Wash twice with PBS (200 μl/well).
  - 3. Block plate by adding 200  $\mu$ l/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-γ standard by reconstituting contents of vial 4 in 200 μl 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 μg/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 μl/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  $\mu$ l/well of mAb rIFN $\gamma$ -II-biotin at 1  $\mu$ g/ml in incubation buffer. Incubate for 1 hour at room temperature.
  - 9. Wash as in step 4.
  - 10. Add 100 μl/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  $\mu$ l/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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Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the following standards:





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