# ELISA for Equine IFN-γ

Product Code: 3117-1A-6

## CONTENTS, development kit for 6 plates:

### Vial 1 (green top)

Monoclonal antibody bIFNγ-I (300 μl)

Concentration: 0.5 mg/ml

# Vial 2 (red top)

Biotinylated monoclonal antibody PAN (40 µl)

Concentration: 0.5 mg/ml

# Vial 3 (white top)

Streptavidin-Alkaline Phosphatase (80 µl)

#### Vial 4

Recombinant equine IFNγ standard

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.

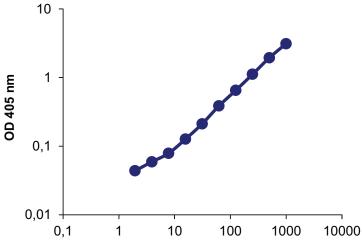
# General

**Intended use:** For quantitative determination of native and recombinant equine IFN- $\gamma$  in solution, e.g. cell culture supernatant and serum/plasma samples. The two mAbs cross react with native ovine IFN- $\gamma$  and native and recombinant bovine IFN- $\gamma$ .

**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: 8-800 pg/ml.

Standard calibration: No international standard exists for calibration



Concentration of equine IFN-y standard (pg/ml)

# Guidelines for Equine IFN-γ ELISA

- **Day 1** 1. Coat a high protein binding ELISA plate with mAb bIFN $\gamma$ -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 equine IFN-γ standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA and leave at room temperature for 15 minutes, then vortex the tube and spin down. This gives a concentration of 0.2 μg/ml. Use immediately or store in aliquots at -20°C for future use. We recommend the aliquots not to 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 PAN-biotin at 0.1  $\mu$ 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 μl/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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