

# Canine IFN- $\gamma$ ELISA development kit

Product Code: 31 13-1H-20

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CONTENTS, development kit for 20 plates:

**Vial 1 (green top)**

Monoclonal antibody MT13 (1000  $\mu$ l)

Concentration: 0.5 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody MT166 (250  $\mu$ l)

Concentration: 0.5 mg/ml

**Vial 3 (white top)**

Streptavidin-Horseradish Peroxidase (250  $\mu$ l)

**Vial 4**

Recombinant canine IFN- $\gamma$  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

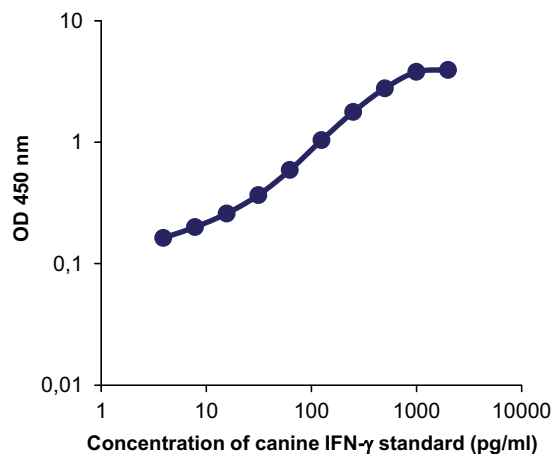
**Intended use:** For quantitative determination of native and recombinant canine IFN- $\gamma$  in solution, e.g. cell culture supernatant and serum/plasma samples.

**Serum/plasma samples:** Please note that cytokine determinations in serum/plasma requires the use of ELISA diluent (product code: 3652-D2) for dilution of samples, standard and detection antibody. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies found in plasma and serum. Please contact Mabtech for further information.

**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu$ m) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.002% Kathon CG.

**Standard range:** 8-800 pg/ml

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



# Guidelines for Canine IFN- $\gamma$ ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb MT13, 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 canine IFN- $\gamma$  standard by reconstituting contents of vial 4 in 1 ml PBS and leave at room temperature for 15 minutes, then vortex the tube and spin down. This gives a concentration of 0.5  $\mu\text{g}/\text{ml}$ . 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  $\mu\text{l}/\text{well}$  of samples or standards diluted in incubation buffer or ELISA diluent for serum/plasma samples and incubate for 2 hours at room temperature.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb MT166-biotin at 0.5  $\mu\text{g}/\text{ml}$  in incubation buffer or ELISA diluent for serum/plasma samples. Incubate for 1 hour at room temperature.
  9. Wash as in step 4.
  10. Add 100  $\mu\text{l}/\text{well}$  of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. **Please note that sodium azide used in buffers will inhibit HRP activity.**
  11. Wash as in step 4.
  12. Add 100  $\mu\text{l}/\text{well}$  of appropriate substrate solution e.g. TMB, available from Mabtech, product code 3652-F10.
  13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

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.**

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

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