

# ELISA for Mouse IFN- $\gamma$

Product Code: 3321-1A-6

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CONTENTS, development kit for 6 plates:

**Vial 1 (green top)**

Monoclonal antibody AN18 (150  $\mu$ l)

Concentration: 1 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody R4-6A2 (80  $\mu$ l)

Concentration: 1 mg/ml

**Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (80  $\mu$ l)

**Vial 4**

Recombinant mouse IFN- $\gamma$  standard (1  $\mu$ 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 mouse 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.

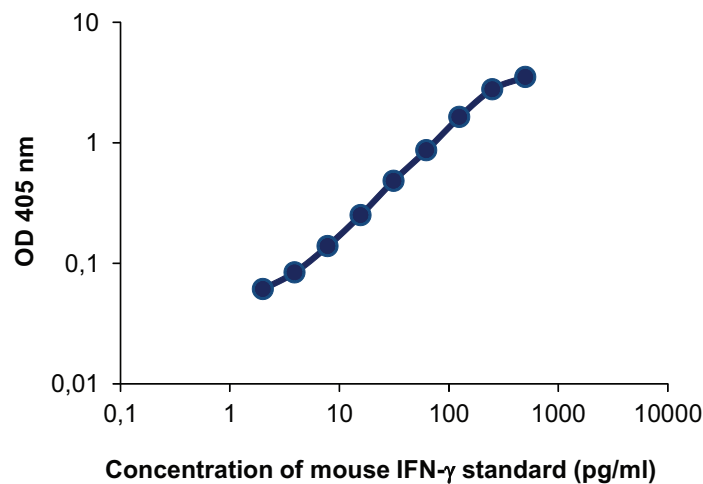
**Standard range:** 4-400 pg/ml

**Limit of detection:** 2 pg/ml

**Intra-assay variation:** < 4%

**Standard calibration:** 1 ng of supplied standard equals 5 U of Gg02-901-533 NIAID\*-standard according to repeated calibrations. Calibration is batch-specific.

\*National Institute of Allergy and Infectious Diseases, USA.



# Guidelines for Mouse IFN- $\gamma$ ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb AN18, diluted to 1  $\mu\text{g}/\text{ml}$  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 mouse IFN- $\gamma$  standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA to a concentration of 1  $\mu\text{g}/\text{ml}$ . Leave at room temperature for 15 minutes and then vortex the tube. 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 R4-6A2-biotin at 0.5  $\mu\text{g}/\text{ml}$  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.



MABTECH AB  
Box 1233  
SE-131 28 Nacka Strand  
Sweden  
Tel: +46 8 716 27 00  
Fax: +46 8 716 27 01  
E-mail: [mabtech@mabtech.com](mailto:mabtech@mabtech.com)  
[www.mabtech.com](http://www.mabtech.com)

MABTECH Inc  
M.E.B. 220  
3814 West Street  
Cincinnati, OH 45227  
USA  
Tel: +1 513 871 4500  
Fax: +1 513 871 7353  
E-mail: [mabtech.usa@mabtech.com](mailto:mabtech.usa@mabtech.com)

MABTECH AB Büro Deutschland  
Germany  
Tel: +49 40 4135 7935  
Fax: +49 40 4135 7945  
E-mail: [mabtech.de@mabtech.com](mailto:mabtech.de@mabtech.com)

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



MABTECH AUSTRALIA Pty Ltd  
resolvingIMAGES  
Unit 22, 196 Settlement Road  
Thomastown Victoria 3074  
Australia  
Tel: +61 3 9466 4007  
Fax: +61 3 9466 4003  
E-mail: [mabtech.au@mabtech.com](mailto:mabtech.au@mabtech.com)

MABTECH AB Bureau de liaison France  
BP 255, 1300 route des Crêtes  
06905 Sophia Antipolis  
France  
Tel: +33 (0)4 92 38 80 70  
Fax: +33 (0)4 92 38 80 71  
E-mail: [mabtech.fr@mabtech.com](mailto:mabtech.fr@mabtech.com)