

# Product Information Sheet

# Mouse IFNy ELISA Kit

Catalog No.	NB-06-0911
Size	96T

Range	31.2pg/ml-2000pg/ml
5	15

Sensitivity < 5 pg/ml

#### Specificity

No detectable cross-reactivity with any other cytokine.

#### Storage

Store at 4 °C for frequent use, at -20 °C for infrequent use. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

#### Expiration

Four months at  $4^{\circ}$ C and eight months at  $-20^{\circ}$ C.

#### Application

For quantitative detection of mouse IFNγ in sera, plasma, body fluids, tissue lysates or cell culture supernates.

#### Principle

Neo Biotech's mouse IFNy ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse IFNv specific-specific polyclonal antibodies were precoated onto 96-well plates. The mouse specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the mouse IFNy amount of sample captured in plate.

#### Kit Components

- 1. Lyophilized recombinant mouse IFNγ standard: 10ng/tube×2.
- 2. One 96-well plate precoated with anti- mouse IFNγ antibody.
- 3. Sample diluent buffer: 30 ml
- 4. Biotinylated anti- mouse IFNγ antibody: 130µl, dilution 1:100.
- 5. Antibody diluent buffer: 12ml.
- 6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
- 7. ABC diluent buffer: 12ml.
- 8. TMB color developing agent: 10ml.
- 9. TMB stop solution: 10ml.

#### Material Required But Not Provided

- 1. Microplate reader in standard size.
- 2. Automated plate washer.
- 3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- 4. Clean tubes and Eppendorf tubes.
- 5. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS:** Add 1.2g Tris, 8.5g Nacl; 450 $\mu$ l of purified acetic acid or 700 $\mu$ l of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride,  $1.4g Na_2HPO_4$  and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

## FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC AND CLINICAL USE.



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## Notice for Application of Kit

- 1. Before using Kit, spin tubes and bring down all components to bottom of tube.
- 2. Duplicate well assay was recommended for both standard and sample testing.
- 3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- 4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

## Mouse IFNy ELISA Kit-1X96 Well Plate Image



## Background

Interferon-gamma (IFN-gamma) is an inflammatory cytokine that has been implicated in the development of fibrosis in inflamed tissues. The production of IFN-gamma, which is under genetic control, can influence the development of fibrosis in lung allografts.<sup>1</sup> IFN-gamma is also produced by natural killer (NK) cells and most prominently by CD8 cytotoxic T cells, and is vital for the control of microbial pathogens.<sup>2</sup> Interferon gamma is believed to be crucial for host defence against many infections. Genetically determined variability in IFN-gamma and expression might be important for the development of tuberculosis.<sup>3</sup> IFN-gamma activates human macrophage oxidative metabolism and antimicrobial activity.<sup>4</sup> In addition to having antiviral activity, IFN-gamma has important immunoregulatory functions. IFN-gamma plays an important role in the control of neointima proliferation.<sup>5</sup> The standard product used in this kit is recombinant mouse IFNγ, consisting of 134 amino acid with the molecular mass of 15.6KDa.

### Reference

- 1. Awad, M.; Pravica, V.; Perrey, C.; El Gamel, A.; Yonan, N.; Sinnott, P. J.; Hutchinson, I. V. CA repeat allele polymorphism in the first intron of the human interferon-gamma gene is associated with lung allograft fibrosis. *Hum. Immunol.* 60: 343-346, 1999.
- Szabo, S. J.; Sullivan, B. M.; Stemmann, C.; Satoskar, A. R.; Sleckman, B. P.; Glimcher, L. H. Distinct effects of T-bet in T(H)1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. *Science* 295: 338-342, 2002.
- 3. Rossouw, M.; Nel, H. J.; Cooke, G. S.; van Helden, P. D.; Hoal, E. G. Association between tuberculosis and a polymorphic NF-kappa-B binding site in the interferon gamma gene. *Lancet* 361: 1871-1872, 2003.
- 4. Nathan, C. F.; Murray, H. W.; Wiebe, M. E.; Rubin, B. Y. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* 158: 670-689, 1983.
- Zohlnhofer, D.; Richter, T.; Neumann, F.-J.; Nuhrenberg, T.; Wessely, R.; Brandl, R.; Murr, A.; Klein, C. A.; Baeuerle, P. A. Transcriptome analysis reveals a role of interferon-gamma in human neointima formation. *Molec. Cell* 7: 1059-1069, 2001.

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