

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# **Product Information**

## Anti-Interferon-B

produced in goat, affinity isolated antibody

Catalog Number I3653

## **Product Description**

Anti-Interferon- $\beta$  (IFN- $\beta$ ) is produced in goat using purified recombinant human IFN- $\beta$  expressed in *E. coli* as immunogen. The antibody is purified using human IFN- $\beta$  affinity chromatography.

Anti-Interferon- $\beta$  reacts with human Interferon- $\beta$  and may be used for neutralization and immunoblotting. Anti-IFN- $\beta$  may be used to neutralize the bioactivity of recombinant human IFN- $\beta$ . It will not block the activity of recombinant human IFN- $\alpha$  or IFN- $\gamma$ . In immunoblotting, the antibody shows no cross-reactivity with recombinant human IFN- $\gamma$ .

The mammalian type I IFNs<sup>1</sup> are produced in response to viral infection and other inducers. They are divided into  $\alpha$  and  $\beta$  subtypes leukocytes and fibroblasts reactivity.<sup>1</sup> The human IFN- $\alpha$ s are encoded by a family of at least 15 different genes, while IFN- $\beta$  is the unique member of its subtype.<sup>2</sup> There is approximately 50% amino acid homology between the  $\alpha$  and  $\beta$  subtypes.<sup>2,3</sup>

Both IFN subtypes are pleiotropic cytokines and have a similar range of biological activities. <sup>3</sup> Differences between  $\alpha$  subtypes and between IFN- $\alpha$  and - $\beta$  are in potency and cell type specific activities. <sup>4</sup> In particular, IFN- $\beta$  elicits a markedly higher antiproliferation response in some cell types such as, <sup>5</sup> embryonal carcinoma, melanoma, and melanocytes than do IFN- $\alpha$ . <sup>6-7</sup> Higher potency of IFN- $\beta$  in treatment of multiple sclerosis and certain cancers has been observed. <sup>7</sup>

Type I IFNs signal through binding to a common cell surface receptor. But and ifnar2, have been identified. Both chains are necessary for function and in the absence of either there is neither high affinity binding nor biological activity. The intracellular portions of the receptor subunits are bound by tyrosine kinases, Jak1 and Tyk2, members of the Janus kinase family. Upon ligand binding these kinases are activated and phosphorylate members of the STAT family of transcription factors, as well as ifnar1 and 2.

## Reagent

Lyophilized from 0.2  $\mu$ m-filtered solution in phosphate buffered saline containing carbohydrates.

#### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## **Preparation Instructions**

To one vial of lyophilized powder, add 1 ml of 0.2  $\mu$ m-filtered PBS to produce a 0.1 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

### Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted product may be stored at 2–8 °C for at least one month. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing

#### **Product Profile**

#### Neutralization of Bioactivity:

To measure the ability of the antibody to neutralize the bioactivity of human IFN- $\beta$ , recombinant human IFN- $\beta$  was incubated with various concentrations of the antibody and added to confluent cultures of HeLa cells. The assay mixture in a total volume of 100  $\mu$ l per well, containing antibody at concentrations of 0.01  $\mu$ g/ml-100  $\mu$ g/ml, recombinant human IFN- $\beta$  at 10 ng/ml was incubated at 37 °C for 20–24 hours in a humidified CO2 incubator. Media was aspirated and an appropriate amount of EMCV was added to each well and incubated at for another 20–24 hours. Cells were then fixed, stained, and scored for cytopathic effect by measurement of optical densities in a microplate reader at 540 nm.  $^{22}$ 

The  $ND_{50}$  is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

Neutralization: using a anti-viral bioassay, a working concentration of 0.05–0.2  $\mu$ g/ml of Anti-IFN- $\beta$  will neutralize 50% of the bioactivity due to 10 ng/ml recombinant human IFN- $\beta$  using HeLa cells.

Immunoblotting: a working concentration of 0.1-0.2  $\mu$ g/ml is determined using human IFN- $\beta$  at 5 ng/lane under non-reducing and reducing conditions.

<u>Note</u>: In order to obtain the best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

#### References

- Stewart, W. E., II, The Interferon System, Springer-Verlag, New York, pp. 135-156, (1979).
- Weissmann, C., and Weber, H., Prog. Nucleic Acid Res., 33, 251-300 (1986).
- Pestka, S. et al., Annu. Rev. Biochem., 56, 727-777 (1987).
- 4. Einhorn, S., and Strander, H., *J. Gen. Virol.*, **35**, 573-577 (1977).
- Rosenblum, M.G. et al., J. Interferon Res., 10, 141-151 (1990).
- Belhumeur, P. et al., Mol. Cell. Biol., 13, 2846-2857 (1993).
- 7. Pfeffer, L. M., and Constantinescu, S. N., in Interferon Therapy in Multiple Sclerosis, Reder, A. T., (ed), Marcel Dekker, New York (1997).

- 8. Branca, A.A., and Baglioni, C., *Nature*, **294**, 768-770 (1981).
- 9. Flores, I. et al., *J. Biol. Chem.*, **266**, 19875-19877 (1991).
- 10. Li, J., and Roberts, R.M., *J. Biol. Chem.*, **269**, 13544-13550 (1994).
- 11. Uzé, G., Lutfalla, G., and Gresser, I., *Cell*, **60**, 225-234 (1990).
- 12. Novick, D. et al., Cell, 77, 391-400 (1994).
- 13. Domanski, P. et al., *J. Biol. Chem.*, **270**, 21606-21611 (1995).
- 14. Lutfalla, G. et al., *EMBO J.*, **14**, 5100-5108 (1995).
- 15. Uzé, G. et al., *J. Interferon Cytokine Res.*, **15**, 3-26 (1995).
- Uzé, G. et al., *Proc. Natl. Acad. Sci. USA*, 89, 4774-4778 (1992).
- 17. Cleary, C.M. et al., *J. Biol. Chem.*, **269**, 18747-18749 (1994).
- 18. Colamonici, O. et al., *Mol. Cell. Biol.*, **14**, 8133-8142 (1994).
- 19. Velazquez, L. et al., Cell, 70, 313-322 (1992).
- 20. Barbieri, G. et al., *Eur. J. Biochem.*, **223**, 427-435 (1994).
- 21. Darnell, J.E. Jr., et al., *Science*, **264**, 1415-1421(1994).
- 22. Meager, A., in Lymphokines and Interferons, a practical approach. Clemens, M.J., et al., (eds.), IRL Press, p 129 (1987).

KAA,PHC,TMS,MAM 06/16-1