

## Product Information

### Anti-Interleukin-10 Receptor $\beta$

produced in goat, affinity isolated antibody

Catalog Number **I3032**

### Synonym: Anti-IL-10 Receptor $\beta$

#### Product Description

Anti-Interleukin-10 Receptor  $\beta$  is produced in goat using as immunogen a purified recombinant human IL-10 R $\beta$  extracellular domain expressed in mouse NSO cells. Affinity isolated antibody is obtained from goat anti-IL-10 R $\beta$  antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Interleukin-10 Receptor  $\beta$  recognizes recombinant human IL-10 R $\beta$  by various immunochemical techniques including ELISA, immunoblotting, neutralization, and flow cytometry.

Reduced human IL-10 R $\beta$  has a calculated molecular mass of ~51 kDa. As a result of glycosylation, the recombinant protein migrates as a 75-85 kDa protein in SDS-PAGE under reducing conditions. Human and mouse interleukin-10 receptor  $\beta$  share approximately 69% amino acid sequence identity.

Interleukin-10, initially named cytokine synthesis inhibitory factor (CSIF), is a potent immunosuppressant of macrophage functions. IL-10, a pleiotropic cytokine, exerts either immunostimulatory or immunosuppressive effects on a number of cell types, including Thy2 cells, activated fetal thymocytes, macrophages, keratinocytes, and LY-1 (CD5<sup>+</sup>) and normal B cells. IL-10 stimulates the growth of stem cells, mast cells, and thymocytes<sup>1</sup> and also enhances cytotoxic T cell development,<sup>2</sup> co-stimulates B cell differentiation, and immunoglobulin secretion.<sup>3</sup>

Interleukin-10 binds specifically and with high affinity to cell-surface receptors. Interleukin-10 receptors are members of the class II subgroup of the cytokine receptor superfamily. Mouse and human cDNA clones encoding the ligand binding IL-10 receptor have been isolated.<sup>4,5</sup> The IL-10 receptor complex contains two distinct type II cytokine receptor subunits, the ligand binding IL-10 R $\alpha$  and the IL-10 R $\beta$ . The co-expression

of both chains is essential for IL-10-induced signal transduction. The  $\beta$  chain serves as an accessory chain essential for the active IL-10 receptor complex and necessary for the initiation of signal transduction.<sup>6</sup>

Interleukin-10 receptors are expressed in all cell types that are known to respond to IL-10. The human interleukin-10 receptor gene maps to chromosome 11q23.3.<sup>7</sup>

#### Reagent

Supplied as 100  $\mu$ g of antiserum lyophilized from a 0.2  $\mu$ m filtered solution of PBS with 5% trehalose.

#### Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline to produce a 0.1 mg/ml stock solution.

#### 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, and storage in "frost-free" freezers.

#### Product Profile

Anti-Interleukin-10 Receptor  $\beta$  has the ability to block human IL-10 receptor  $\beta$  mediated IL-10 response on lipopolysaccharide (LPS)-activated PBMCs (peripheral blood mononuclear cells). PBMCs are added to a 96 well plate containing various concentrations (0.01-200  $\mu$ g/ml) of the antibody and incubated for 1 hour at 37 °C. After this pre-incubation, recombinant human IL-10 and LPS (lipopolysaccharide) are added. The assay mixture in a total volume of 200  $\mu$ l, containing antibody (concentrations of 0.01-200  $\mu$ g/ml), LPS (0.25 ng/ml), recombinant human IL-10 (0.25 ng/ml), and cells (1.5 x 10<sup>6</sup>/ml), is incubated at 37 °C for 24 hours in a humidified CO<sub>2</sub> incubator. After the incubation, 25  $\mu$ L of the supernatant is collected from each well, diluted at 1:4, and assayed for IL-1 $\beta$  levels using an IL-1 $\beta$  ELISA.

The Neutralization Dose<sub>50</sub> (ND<sub>50</sub>) for Anti-IL-10 R $\beta$  is 2-6  $\mu$ g/ml in the presence of 0.25 ng/ml of recombinant human IL-10 using human PBMCs.

The ND<sub>50</sub> is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize human cell surface IL-10 R $\beta$  mediated bioactivity is dependent on the IL-10 concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working concentration of 0.1-0.2  $\mu$ g/ml is recommended. The detection limit for recombinant human IL-10 R $\beta$  is ~5 ng/lane and 2 ng/lane under non-reducing and reducing conditions, respectively.

ELISA: a working concentration of 0.5-1.0  $\mu$ g/ml is recommended. The detection limit for recombinant human IL-10 R $\beta$  is ~0.03 ng/well.

Flow cytometry: 25-50  $\mu$ g/ml (10  $\mu$ L/10<sup>5</sup> cells) is recommended to detect human IL-10 R $\beta$ .

**Note:** In order to obtain the best results in various techniques and preparations we recommend determining the optimal working dilutions by titration.

Endotoxin level; <20 ng/mg antibody as determined by the LAL (Limulus amoebocyte lysate) method.

#### References

1. Rennick, D., et al., *Progress in Growth Factor Research*, **4**, 207 (1992).
2. Chen, W., et al., *J. Immunol.*, **147**, 528 (1991).
3. Rousset, F., et al., *Proc. Natl. Acad. Sci. USA*, **89**, 1890 (1992).
4. Liu, Y., et al., *J. Immunol.*, **152**, 1821-1829 (1994).
5. Ho, A.S-Y., et al., *Proc. Natl. Acad. Sci. USA*, **90**, 11267-11271 (1993).
6. Kotenko, S.V., et al., *EMBO J.*, **16** (5894-5903 (1997).
7. Taniyama, T., et al., *Hum. Genet.*, **95**, 99-101 (1995).

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