

## Product Information

### Monoclonal Anti-Interleukin-10 Receptor $\alpha$ clone 37607

antibody produced in mouse, purified immunoglobulin

Catalog Number **I3282**

#### Product Description

Monoclonal Anti-Human Interleukin-10 Receptor  $\alpha$  (mouse IgG1 isotype) is produced from a mouse hybridoma elicited from a mouse immunized with purified recombinant human interleukin 10 soluble receptor  $\alpha$ , expressed in insect *Sf* 21 cells. The antibody is purified from the IgG fraction of ascities fluid using Protein G chromatography.

Monoclonal Anti-Human Interleukin-10 Receptor  $\alpha$  (IL-10 R $\alpha$ ) recognizes recombinant human IL-10 R $\alpha$ . It can be used in neutralization applications. The antibody does not cross-react with recombinant mouse IL-10 R $\alpha$ .

Recombinant human IL-10 R $\alpha$  (214 amino acids), generated after cleavage of a 21 amino acid residue signal peptide, has a predicted molecular mass of 24 kDa. As a result of glycosylation, the recombinant protein migrates as ~32 kDa protein in SDS-PAGE. Human and mouse interleukin-10 receptors share ~60% 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 lyophilized from a 0.2  $\mu$ m filtered solution of phosphate buffered saline with 5% trehalose.

#### Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline to produce 0.5 mg/ml stock solution of antibody.

#### Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted antibody may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

#### Product Profile

Monoclonal Anti-Human Interleukin-10 Receptor  $\alpha$  has the ability to block human IL-10 receptor  $\alpha$  mediated IL-10 response on lipopolysaccharide (LPS)-activated PBMCs (peripheral blood mononuclear cells). PBMCs are added to a 96 well plate containing various concentrations (0.001-10  $\mu$ g/ml) of the antibody and incubated for 1 hour at 37 °C. Following this pre-incubation, recombinant human IL-10 and LPS are added. The assay mixture in a total volume of 200  $\mu$ L, containing antibody (concentrations of 0.001-10  $\mu$ 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, 150  $\mu$ L of the supernatant is collected from each well 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-human IL-10 R $\alpha$  is 0.03-0.1  $\mu$ 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 cell surface IL-10 R $\alpha$ -mediated IL-10 response on a responsive cell line, at a specific IL-10 concentration.

The exact concentration of antibody required to neutralize human cell surface IL-10 R $\alpha$  mediated bioactivity is dependent on the IL-10 concentration and the number of IL-10 R $\alpha$ 's present on the cell surface (a function of cell type and culture conditions).

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

Endotoxin level is <10 ng/mg antibody as determined by the LAL (Limulus amoebocyte lysate) method.

#### References

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7. Taniyama, T., et al., *Hum. Genet.*, **95**, 99-101 (1995).

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