

## Product Information

### **Monoclonal Anti-Interleukin-2, clone 5334**

produced in mouse, purified immunoglobulin

Catalog Number **I6784**

#### **Product Description**

Monoclonal Anti-Interleukin-2 (IL-2) (mouse IgG1 isotype) is purified from a hybridoma produced by the fusion of mouse myeloma cells and B cells from a mouse immunized with recombinant human Interleukin-2 (GenelD 3558) expressed and purified from *Escherichia coli*. The antibody is purified by Protein G affinity chromatography.

Monoclonal Anti-Interleukin-2 recognizes human Interleukin-2. Applications include immuno-histochemistry, flow cytometry, and neutralization. This antibody detects natural and recombinant human Interleukin-2. This antibody will not neutralize the biological activity of recombinant mouse Interleukin-2. When immobilized on a microplate, this antibody will capture recombinant and natural human IL-2.

Interleukin-2 is a T cell-derived cytokine also called T cell growth factor (TCGF). It stimulates the growth and differentiation of T cells, B cells, NK cells, LAK cells, monocytes, macrophages and oligodendrocytes.<sup>1-3</sup> It functions through the heterotrimeric IL2 receptor comprising  $\alpha$ ,  $\beta$ , and  $\gamma$  chains. There is 60% homology between human and mouse IL2.

#### **Reagent**

Supplied lyophilized from a 0.2  $\mu$ m filtered solution of phosphate buffered saline with 5% trehalose.

#### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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 0.5 mg/mL stock solution. If aseptic technique is used, no further filtration should be necessary for use in cell culture environments.

#### **Storage/Stability**

Prior to reconstitution, store at  $-20^{\circ}\text{C}$ . Reconstituted product may be stored at  $2-8^{\circ}\text{C}$  for up to one month. For extended storage, freeze in working aliquots at  $-20^{\circ}\text{C}$ . Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended.

#### **Neutralization**

To measure the ability of the antibody to neutralize the bioactivity of recombinant human Interleukin-2 on CTLL-2 cells, recombinant human Interleukin-2 was incubated with various concentrations of the antibody for 1 hour at  $37^{\circ}\text{C}$  in a 96 well plate. Following this preincubation period, CTLL-2 cells were added. The assay mixture in a total volume of 100  $\mu$ L, containing antibody at 0.001-10  $\mu$ g/mL, recombinant human Interleukin-2 at 2 ng/mL, and cells at  $1 \times 10^5$  cells/mL, was incubated at  $37^{\circ}\text{C}$  for 24 hours in a humidified  $\text{CO}_2$  incubator.  $^3\text{H}$ -thymidine was added during the final 4 hours of incubation. The cells were harvested onto glass fiber filters and the  $^3\text{H}$ -thymidine incorporated into DNA was determined.

The Neutralization Dose<sub>50</sub> (ND<sub>50</sub>) for this antibody is defined as that 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.

#### **Product Profile**

Immunohistochemistry: a working concentration of 8-25  $\mu$ g/mL is recommended to detect recombinant human Interleukin-2 in activated human peripheral blood leukocytes (PBLs).

**Flow Cytometry:** this antibody may be used for intracellular staining to detect human Interleukin-2. Cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in phosphate buffered saline. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to  $1-5 \times 10^5$  cells in a total reaction volume not exceeding 200 µL. Following a 30 minute incubation, cells should be washed with 0.1% saponin prior to addition of a secondary developing reagent. The binding of unlabeled monoclonal antibodies may be visualized by adding 10 µL of a 25 µg/mL solution of a secondary developing reagent such as goat Anti-mouse IgG conjugated to a fluorochrome. Cells should be washed for a final time in 0.1% saponin prior to flow cytometric analysis.

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

Endotoxin: <0.1 EU/µg antibody as determined by the LAL method.

#### References

1. Smith, K., *Ann. Rev. Immunol.*, **2**, 319 (1984).
2. Smith, K., *Science*, **240**, 1169 (1988).
3. Kuziel, W., et al., *The Cytokine Handbook*, Thomson. A. (ed.), Academic Press, London, 83 (1991).

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