

## Product Information

### ANTI-FLT-3/FLK-2 LIGAND (FL), Mouse Developed in Goat, Affinity Isolated Antibody

Product Number **F 8925**

#### Product Description

Anti-Mouse Flt-3/Flk-2 Ligand (FL) is developed in goat using a purified recombinant mouse Flt-3 ligand expressed in mouse NSO cells as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-Flt-3 ligand antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Mouse Flt-3/Flk-2 Ligand (FL) recognizes recombinant mouse Flt-3 ligand by various immunochemical techniques including neutralization, immunoblotting, and ELISA. Based on ELISA, this antibody shows less than 10 % cross-reactivity with recombinant human Flt-3 ligand.

Recombinant mouse Flt-3/Flk-2 Ligand (FL) is produced from a DNA sequence encoding the extracellular domain of mouse FL (amino acid residues 1 to 188).<sup>1</sup> The 162 amino acid residue protein, generated after removal of the 26 amino acid residue signal peptide, has a predicted molecular mass of approximately 18 kDa. The gene for FL has been mapped to chromosome 7 in the mouse.

The Flt-3 (*fms*-like tyrosine kinase-3)/Flk-2 (fetal liver kinase-2) Ligand (FL), a hematopoietic cytokine, was cloned based on its ability to bind and activate *c-kit* and Flt-3 tyrosine kinase receptors.<sup>2</sup> Fl plays a key role in hematopoiesis by stimulating proliferation and/or differentiation of various hematopoietic cell types *in vitro* as well as *in vivo*.<sup>3,4</sup> Mouse FL, a type I trans-membrane glycoprotein, can undergo proteolytic cleavage to generate a soluble form of the protein. Also, an alternatively spliced FL mRNA, encoding a soluble form of the mouse FL, has been identified. Mouse and human FL share 72 % amino acid sequence homology and exhibit full species cross-reactivity. Both the transmembrane and soluble forms of FL are biologically active.

FL has been shown to synergize with a wide variety of hematopoietic cytokines to stimulate the growth and differentiation of early hematopoietic progenitors. FL promotes growth of early B cell progenitor cells in combination with IL-7<sup>5</sup> and induces adhesion of the precursor B cell line BaF3/Flt3 to fibronectin by activating the fibronectin receptors VLA-4 and VLA-5 integrins.<sup>6</sup>

Flt-3/Flk-2 ligand is widely expressed in a variety of mouse tissues. Cells known to express FL include T cell lines<sup>1</sup>, a thymic stromal cell line<sup>7</sup>, bone marrow fibroblasts<sup>8</sup>, and hematopoietic cells. Flt-3 receptor is expressed in a variety of tissues including placenta, gonads, and tissues of nervous and hematopoietic origin. In the hematopoietic system, the expression of Flt-3/Flk-2 ligand and Flt-3 receptor is restricted to the population enriched for precursor/progenitor cells.<sup>9</sup> The gene for FL has been mapped to chromosome 7 in the mouse.

#### Reagent

Anti-Mouse Flt-3/Flk-2 Ligand (FL) is supplied as 100 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline (PBS).

#### Preparation Instructions

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

#### Storage/Stability

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

### Product Profile

Anti-Mouse Flt-3/FIk-2 Ligand (FL) has the ability to neutralize the biological activity of recombinant mouse Flt-3 ligand on murine Bafl1 cell. Recombinant mouse FLT-3 ligand is added to various concentrations of the antibody for 1 hour at 37 °C in a 96 well microplate. Following this pre-incubation, Bafl1 cells are added. The assay mixture in a total volume of 100 µl, containing antibody at concentrations of 0.001 µg/ml to 7 µg/ml, recombinant mouse Flt-3 ligand at 25 ng/ml, and cells at 1 x 10<sup>5</sup> cells/ml, is incubated at 37 °C for 40 to 48 hours in a humidified CO<sub>2</sub> incubator. The mixture is pulsed with <sup>3</sup>H-thymidine during the final 4 hours. The cells are detached and harvested onto glass fiber filters, and the <sup>3</sup>H-thymidine incorporated into the DNA is measured.<sup>6</sup>

The Neutralization Dose<sub>50</sub> (ND<sub>50</sub>) for anti-mouse Flt-3 ligand is approximately 0.2 to 0.6 µg/ml in the presence of 25 ng/ml of recombinant mouse Flt-3 ligand using the mouse Bafl1 cells.

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 the cytokine is present at a concentration just high enough to elicit a maximum response.

Note: The exact concentration of antibody required to neutralize mouse Flt-3 ligand activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

For immunoblotting, a working concentration of 0.1 to 0.2 µg/ml antibody is recommended. The detection limit for recombinant mouse Flt-3 ligand is approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working concentration of 0.5 to 1.0 µg/ml antibody is recommended. The detection limit for recombinant mouse Flt-3 ligand is approximately 0.06 ng/well.

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

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

### References

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