

## Reagents Provided

### Alexa Fluor® 700-conjugated mouse monoclonal anti-human

**Flt-3/Flk-2:** Supplied as 25 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

**Clone #:** 66903

**Isotype:** mouse IgG<sub>1</sub>

## Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

## Storage

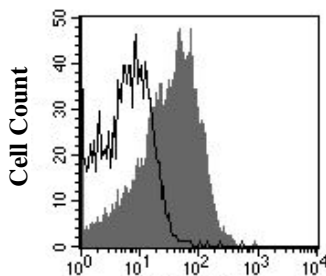
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing Flt-3/Flk-2 within a population and qualitatively determine the density of Flt-3/Flk-2 on cell surfaces by flow cytometry.

## Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified NS0-derived recombinant human Flt-3/Flk-2 (aa 27-541; Accession # AAA18947). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 700 fluorochrome. Cell surface expression of Flt-3/Flk-2 is determined by flow cytometry using 675-700 nm wavelength excitation range and monitoring emitted fluorescence with a detector optimized to collect peak emissions at around 723 nm.



**Flt-3/Flk-2-Alexa Fluor® 700**

Human THP-1 cells were stained with Alexa Fluor® 700-conjugated anti-human Flt-3/Flk-2 (Catalog # FAB812N; filled histogram) or Alexa Fluor® 700-conjugated isotype control (Catalog # IC002N; open histogram).

## Legal

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## Background Information

The Flt-3 (*fms*-like tyrosine kinase) receptor, also named Flk-2 (fetal liver kinase) and Stk-1 (stem cell tyrosine kinase) is a member of the class III subfamily of receptor tyrosine kinases that also includes SCF R/c-kit, the receptor for SCF and M-CSF R/FMS, the receptor for M-CSF. The extracellular region of these receptors contains five immunoglobulin-like domains and the intracellular region contains a split kinase domain. Human Flt-3 cDNA encodes a 993 amino acid (aa) residue type I membrane protein with a 26 aa residue signal peptide, a 515 aa extracellular domain with 10 potential N-linked glycosylation sites, a 21 aa residue transmembrane domain and a 431 aa residue cytoplasmic domain. Mouse Flt-3 has also been cloned and shown to share 85% amino acid sequence identity with human Flt-3. Flt-3 expression has been detected in various tissues, including placenta, gonads, and tissues of nervous and hematopoietic origin. Among hematopoietic cells, the expression of Flt-3 was found to be restricted to the highly enriched stem/progenitor cell populations. The ligand for Flt-3 has been identified to be a transmembrane protein with structural homology to M-CSF and SCF. Recombinant soluble Flt-3/Fc chimeric protein has been shown to bind Flt-3 Ligand with high affinity and is a potent Flt-3 Ligand antagonist.

## References

- Rosnet, O. *et al.* (1996) *Acta. Haematol.* **95**:218.
- Drexler, H.G. (1996) *Leukemia* **10**:588.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using human THP-1 cells.

- Cells may be Fc-blocked with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 5 µL of conjugated antibody was added to up to 1 x 10<sup>6</sup> cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 700-labeled mouse IgG<sub>1</sub> antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

**Warning:** Contains sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.