

**DESCRIPTION**

<b>Source</b>	<i>E. coli</i> -derived human Flt-3 Ligand/FLT3L protein Thr27-Ala181 Accession # P49771.1 Produced in an animal-free laboratory. Manufactured and tested under cGMP guidelines
<b>N-terminal Sequence Analysis</b>	Met-Thr27-Gln-Asp-(Cys)-Ser-Phe-Gln-His-Ser and Thr27-Gln-Asp-(Cys)-Ser-Phe-Gln-His-Ser-Pro
<b>Predicted Molecular Mass</b>	18 kDa

**SPECIFICATIONS**

<b>SDS-PAGE</b>	15-17 kDa, under reducing conditions
<b>Activity</b>	Measured in a cell proliferation assay using BaF3 mouse pro-B cells transfected with mouse Flt-3. The ED <sub>50</sub> for this effect is 0.2-1 ng/mL.  The specific activity of Recombinant Human Flt-3 Ligand/FLT3L is >5.0 x 10 <sup>5</sup> units/mg, which is calibrated against the human Flt-3 Ligand WHO Standard (NIBSC code: 96/532).
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
<b>Host Cell Protein</b>	< 0.5 ng per µg of protein when tested by ELISA.
<b>Mycoplasma</b>	Negative when tested in a ribosomal RNA hybridization assay.
<b>Host Cell DNA</b>	< 0.0010 ng per µg of protein when tested by PCR.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 100-500 µg/mL in PBS.
<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• A minimum of 6 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 3 months, ≤ -20 °C under sterile conditions after reconstitution.</li> </ul>

**DATA**

**Bioactivity**

**Recombinant Human Flt-3 Ligand/FLT3L GMP Protein Bioactivity** GMP-grade Recombinant Human Flt-3 Ligand/FLT3L (Catalog # 308E-GMP) stimulates cell proliferation in the BaF3 mouse pro-B cell line transfected with mouse Flt-3. The ED<sub>50</sub> for this effect is 0.2-1 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP Flt-3 Ligand/FLT3L.

**SDS-PAGE**

**Recombinant Human Flt-3 Ligand/FLT3L GMP Protein SDS-PAGE** 2 µg/lane of GMP-grade Recombinant Human Flt-3 Ligand/FLT3L (Catalog # 308E-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® blue staining, showing bands at 15-17 kDa and 12-14 kDa, respectively.

**BACKGROUND**

Flt-3 Ligand, also known as FLT3L, is an alpha-helical cytokine that promotes the differentiation of multiple hematopoietic cell lineages (1-3). Mature human Flt-3 Ligand consists of a 158 amino acid (aa) extracellular domain (ECD) with a cytokine-like domain and a juxtamembrane tether region, a 21 aa transmembrane segment, and a 30 aa cytoplasmic tail (4-7). Within the ECD, human Flt-3 Ligand shares 71% and 65% aa sequence identity with mouse and rat Flt-3 Ligand, respectively (4-6). The human and mouse Flt-3 Ligand proteins show cross-species activity. Flt-3 Ligand is also structurally related to M-CSF and SCF. Flt-3 Ligand is widely expressed in various human and mouse tissues. It is expressed as a noncovalently-linked dimer by T cells and bone marrow and thymic fibroblasts (1, 8). Each 36 kDa chain of the Flt-3 Ligand dimer carries approximately 12 kDa of N- and O-linked carbohydrates (8). Alternate splicing and proteolytic cleavage of the transmembrane form of the Flt-3 Ligand protein can generate a soluble 30 kDa fragment that includes the cytokine-like domain (4, 8). Alternate splicing of human Flt-3 Ligand also generates membrane-associated isoforms that contain either a truncated cytoplasmic tail or an 85 aa substitution following the cytokine-like domain in the ECD of the Flt-3 Ligand protein (4, 5, 8). Both transmembrane and soluble forms of Flt-3 Ligand signal through the tyrosine kinase receptor Flt-3/Flk-2 (3, 4, 6, 7). Flt-3 Ligand induces the expansion of monocytes and immature dendritic cells as well as early B cell lineage differentiation (2, 9). Additionally, Flt-3 Ligand synergizes with IL-3, GM-CSF, and SCF to promote the mobilization and myeloid differentiation of hematopoietic stem cells (4-6). Flt-3 Ligand also cooperates with IL-2, IL-6, IL-7, and IL-15 to induce NK cell development and with IL-3, IL-7, and IL-11 to induce terminal B cell maturation (1, 10). Animal studies show that Flt-3 Ligand reduces the severity of experimentally induced allergic inflammation (11).

**References:**

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3. Gilliland, D.G. and J.D. Griffin (2002) *Blood* **100**:1532.
4. Hannum, C. *et al.* (1994) *Nature* **368**:643.
5. Lyman, S.D. *et al.* (1994) *Blood* **83**:2795.
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7. Savvides, S.N. *et al.* (2000) *Nat. Struct. Biol.* **7**:486.
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## MANUFACTURING SPECIFICATIONS

### GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

R&D Systems' quality focus includes:

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- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- N-terminal amino acid analysis, SDS-PAGE analysis, mass spectrometry, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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- Dedicated fermentors are utilized in committed animal-free areas.

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- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

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