

Product Information

LIGHT

Human, Recombinant
Expressed in NSO mouse myeloma cells

Product Number **L 0414**
Storage Temperature -20°C

Synonyms: TNFSF14, HVEM, LT γ

Product Description

LIGHT is a 240 amino acid type II transmembrane protein consisting of a 37 amino acid cytoplasmic domain, a 22 amino acid transmembrane domain, and a 181 amino acid extracellular domain. Recombinant LIGHT is produced from a cDNA sequence encoding the extracellular region of human LIGHT (amino acid residues 74-240) that was fused via a polypeptide linker at its amino terminus to a polyhistidine tag and the CD33 signal peptide (Met¹-Met¹⁷).¹ It is expressed in NSO mouse myeloma cells and purified by affinity chromatography. Based on amino acid sequence analysis, the amino terminus of the mature fusion protein is Met¹⁷ of the CD33 signal peptide. The calculated molecular mass of the LIGHT fusion protein is 20.9 kDa. However, as a result of glycosylation, the recombinant protein migrates as a 25 kDa protein in SDS-PAGE under reducing conditions. LIGHT is predicted to assemble as a homotrimer *in vivo*.

LIGHT is a member of the TNF superfamily. The acronym LIGHT stands for "is homologous to Lymphotoxins, exhibits inducible expression, and competes with *Herpes simplex virus* (HSV) glycoprotein D for HVEM, a receptor expressed by T lymphocytes". LIGHT is a ligand for HVEM (Herpes virus entry mediator), a member of the TNF receptor superfamily^{2,3} and for the lymphotoxin β receptor (LT β R).⁴ Binding to these receptors induces apoptosis in various tumor cell lines, including HT29 cells. In contrast, LIGHT can also bind to the decoy death receptor DcR3, that suppresses LIGHT-mediated cytotoxicity.⁵

Overexpression of LIGHT in tumor cells induces apoptosis. The reactivity can be enhanced by Interferon- γ (IFN γ). Evidence suggests that LIGHT influences dendritic cell (DC) maturation and enhances the DC-mediated immune response by its co-stimulatory effect with CD154 (CD40 ligand).^{3,6,7}

Reagents

Recombinant human LIGHT is lyophilized from a sterile filtered phosphate-buffered saline (PBS) solution containing 50 μg bovine albumin (BSA) per 1 μg of LIGHT.

Preparation Instructions

Reconstitute the vial contents with sterile PBS containing at least 0.01% human or bovine albumin (HSA or BSA.). Stock solution concentration should be no less than 50 $\mu\text{g}/\text{ml}$.

Storage/Stability

Lyophilized human recombinant LIGHT is stable for at least six months at -20°C . Upon reconstitution, store the stock solution at $2-4^{\circ}\text{C}$ for up to one month. For extended storage, store in working aliquots at -20°C . Repeated freeze-thaw cycles should be avoided. Do not store in a frost-free freezer.

Product Profile

LIGHT activity is measured in a cytotoxicity assay using HT29 cells in the presence of 10 U/ml IFN- γ . Recombinant human LIGHT causes a 2–4.5-fold inhibition of HT29 cell proliferation at 10 ng/ml. Optimal dilutions should be determined by each laboratory for each application.

Purity: >95% by SDS-PAGE visualized by silver stain.

Endotoxin level: < 0.1 ng/ μg of protein as determined by the LAL (Limulus amoebocyte lysate) method.

References

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2. Montgomery, R., et al., Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell*, 87, 427 – 436 (1996).

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4. Rooney, I.A., et al., The lymphotoxin- β receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells. *J. Biol. Chem.*, **275**, 14307-14315 (2000).
5. Yu, K.Y., et al., A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. *J. Biol. Chem* **274**, 13733-13736 (1999).
6. Morel, Y. et al., Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. *J. Immunol.*, 165, 4397-4404 (2000).
7. Morel, Y., et al., The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. *J. Immunol.*, 167, 2479 – 2487 (2001).

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