

Product Information

Erythropoietin Soluble Receptor human
recombinant, expressed in mouse NSO cells

Catalog Number **E0643**

Synonym: EPO sR

Product Description

Recombinant Erythropoietin Soluble Receptor human is a type I membrane-spanning protein, expressed in a mouse myeloma cell line, NSO. This product is produced from a cDNA sequence encoding the signal peptide from human CD33 and fused to the extracellular domain of the human erythropoietin receptor (Ala 25 - Pro 250).¹ The protein is purified by sequential chromatography. The 225 amino acid residue mature recombinant soluble human EPO receptor, generated after cleavage of a 25 amino acid residue signal peptide, has a predicted molecular mass of ~25 kDa. From glycosylation, recombinant human EPO sR migrates as a ~32 kDa protein in SDS-PAGE.

Erythropoietin (EPO), a glycoprotein produced primarily by the kidney and at lower levels by the liver, is the primary regulatory factor of erythropoiesis.^{2,3} It promotes the proliferation, differentiation, and survival of the erythroid progenitors. The biological effects of EPO are mediated by the erythropoietin receptor (EPO R). The EPO receptor belongs to the cytokine receptor superfamily, which includes the receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, LIF, and others.^{4,5} These receptors contain four conserved cysteines and a Trp-Ser-X-Trp-Ser (WSXWS) motif in their extracellular domains, where X represents any amino acid. The EPO receptor differs from the other members of the cytokine receptor superfamily in that the EPO receptor can be stimulated to signal cell growth by binding either EPO or gp55.⁶ Human and mouse EPO receptor share 82 % identity in their amino acid sequence.⁴ Truncated and soluble forms of the EPO receptor result from alternative splicing of the EPO receptor gene. The gene for human EPO receptor has been mapped to the p region of chromosome 19.⁷

Recombinant soluble EPO receptor binds EPO with high affinity and is a potent EPO antagonist. When EPO is present at low concentrations, the EPO receptor initiates prolongation of G1 of the cell cycle and sends a differentiation signal; whereas at high EPO concentrations, a proliferation signal is generated and the G1 is shortened.⁸ EPO binding induces the stimulation of Jak2 tyrosine kinase, which leads to tyrosine phosphorylation of several proteins, including the EPO receptor, resulting in the activation of intracellular pathways (Ras/MAP kinase, phosphatidylinositol 3-kinase, and STAT transcription factors).^{6,9,10}

Recombinant Erythropoietin Soluble Receptor human is expressed in megakaryocytes, erythroid progenitors, endothelial cells, and, possibly, neurons.⁴ It has also been found in human sera. Expression of the EPO receptor in normally IL-3-dependent cell lines such as Ba/F3 (pro-B lymphocyte cell line) or 32D (myeloid progenitor cell line) allows cell proliferation in the presence of either EPO or IL-3, indicating that the EPO receptor is capable of generating a proliferative signal in these cells.^{4,6}

Reagents

The product is supplied as ~50 µg of protein lyophilized from a 0.2 µm filtered solution of phosphate buffered saline containing 5% trehalose and 50 µg of bovine serum albumin per 1 µg of cytokine.

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

Reconstitute the contents of the vial using sterile phosphate buffered saline containing 0.1% human serum albumin or bovine serum albumin. Prepare a stock solution of no less than 50 µg/mL.

Storage/Stability

Store at -20 °C. Upon reconstitution, the product may be stored at 2-8 °C for one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Do not store in a frost-free freezer.

Product Profile

Recombinant Erythropoietin Soluble Receptor human is measured by its ability to inhibit the recombinant human erythropoietin mediated cell proliferation of human TF1 cells (immature erythroid cells).¹¹

The ED₅₀ is defined as the effective concentration of receptor that elicits a 50% inhibition in cell growth in a cell based bioassay.

Purity: > 90% as determined by SDS-PAGE, visualized by silver stain.

Endotoxin: < 1 EU per 1 µg of protein determined by the LAL method.

References

1. Jones, S.S., et al., Human erythropoietin receptor: cloning, expression, and biologic characterization. *Blood*, **76**, 31-35 (1990).
2. Constantinescu, S.N., et al., The Erythropoietin Receptor: Structure, Activation and Intracellular Signal Transduction. *Trends Endocrinol. Metab.*, **10**, 18-23 (1999).
3. Wojchowski, D.M., et al., Signal transduction in the erythropoietin receptor system. *Exp. Cell Res.*, **253**, 143-156 (1999).
4. Youssoufian, H., et al., Structure, Function, and Activation of the Erythropoietin Receptor. *Blood*, **81**, 2223-2236 (1993).
5. Lodish, H.F., et al., The Erythropoietin Receptor: Biogenesis, Dimerization, and Intracellular Signal Transduction. Cold Spring Harbor Laboratory Press, **60**, 93-104 (1995).
6. Barber, S.L., and D'Andrea, A.D., The Erythropoietin Receptor and the Molecular Basis of Signal Transduction. *Seminars in Hematology*, **29**, 293-304 (1992).
7. Winkelmann, J.C., et al., The gene for the human erythropoietin receptor: Analysis of the coding sequence and assignment to chromosome 19p. *Blood*, **76**, 24-30 (1990).
8. Carroll, M., et al., Erythropoietin-induced cellular differentiation requires prolongation of the G1 phase of the cell cycle. *Proc. Natl. Acad. Sci. USA*, **92**, 2869-2873 (1995).
9. Lacombe, C., and Mayeux, O., *Biology of erythropoietin. Haematologica*, **83**, 724-732 (1998).
10. Heim, M.H., The Jak-STAT pathway: cytokine signaling from the receptor to the nucleus. *J. Recept. Signal. Transduct. Res.*, **19**, 75-120 (1999).
11. Kitamura, T., et al., Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. *J. Cell Physiol.*, **140**, 323-334 (1989).

AH,KAA,PHC 02/10-1