Recombinant Human SCF/c-kit Ligand

Catalog Number: 255B-GMP

DESCRIPTION

Source	*E. coli*-derived human SCF/c-kit Ligand protein Glu26-Ala189, with an N-terminal Met. Accession # P21583.1 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Met-Glu$_{26}$-Gly-Ile-(Cys)-Arg-Asn-Arg-Val-Thr
Predicted Molecular Mass	19 kDa

SPECIFICATIONS

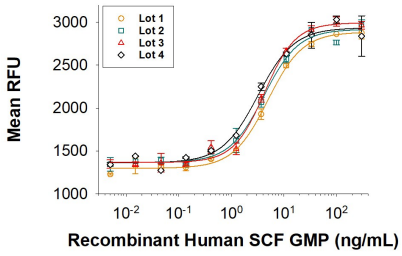
SDS-PAGE	19 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using TF-1 human erythroleukemic cells. Kitamura, T. *et al.* (1989) J. Cell Physiol. **140**:323. The ED$_{50}$ for this effect is 1-5 ng/mL. The specific activity of recombinant human SCF is approximately 564 units/µg, which is calibrated against recombinant human SCF WHO Standard (NIBSC code: 91/682).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Mass Spectrometry	The result from mass spectrometry confirms the major peak corresponds to SCF/c-kit ligand. See the CofA for additional information.
Host Cell Protein	< 0.1 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in PBS.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

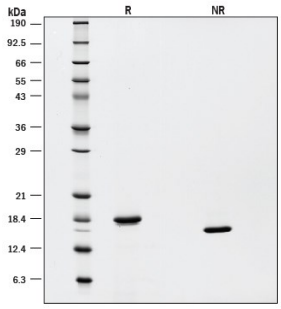
DATA

Bioactivity



GMP-grade Recombinant Human SCF (Catalog # 255B-GMP) stimulates cell proliferation of the TF-1 human erythroleukemic cell line. The ED$_{50}$for this effect is 1-5 ng/mL. Four independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP SCF.

SDS-PAGE



2 µg/lane of GMP-grade Recombinant Human SCF (Catalog # 255B-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing R band at 18 kDa and NR band at 16 kDa, respectively.

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BACKGROUND

Stem cell factor (SCF), also known as c-kit ligand (KL), mast cell growth factor (MGF), and steel factor (SLF), is a widely expressed 28-40 kDa type I transmembrane glycoprotein (1). It promotes the survival, differentiation, and mobilization of multiple cell types including myeloid, erythroid, megakaryocytic, lymphoid, germ cell, and melanocyte progenitors (1-7). SCF is a primary growth and activation factor for mast cells and eosinophils (8). Mature human SCF consists of a 189 amino acid (aa) extracellular domain (ECD), a 23 aa transmembrane segment, and a 36 aa cytoplasmic tail (9). The ECD shows both N-linked and O-linked glycosylation (10). Proteolytic cleavage at two alternate sites in the extracellular juxtamembrane region releases a 25 kDa soluble molecule which is comparable to the only form produced by Steel-dickie mutant mice (11, 12). An alternately spliced isoform of human SCF lacks 28 aa that encompasses the primary proteolytic recognition site (13). Within the ECD of the long isoform (corresponding to this recombinant protein), human SCF shares 79%-87% aa sequence identity with canine, feline, mouse, and rat SCF. Rat SCF is active on mouse and human cells, but human SCF is only weakly active on mouse cells (9). Noncovalent dimers of transmembrane or soluble SCF interact with the receptor tyrosine kinase SCF R/c-kit to trigger receptor dimerization and signaling (14). SCF assists in the recovery of cardiac function following myocardial infarction by increasing the number of cardiomyocytes and vascular channels (15).

References:

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- 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 bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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