Recombinant Human LR3 IGF-I GMP

Catalog Number: 8335-GMP

DESCRIPTION

Source

E. coli-derived

MFPAMPLSSLFVN	Human LR3 IGF-I (Gly49-Ala118 (Glu51Arg)) Accession # P05019
N-terminus	C-terminus

Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.

N-terminal Sequence Analysis	Met-Phe-Pro-Ala-Met-Pro-Leu-Ser-Ser-Leu
Predicted Molecular Mass	9 kDa

SPECIFICATIONS

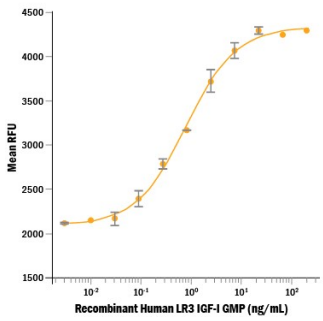
SDS-PAGE	7 kDa, reducing conditions
Activity	Measured in a serum-free cell proliferation assay using MCF-7 human breast cancer cells. Karey, K.P. *et al.* (1988) Cancer Research **48**:4083. The ED_{50} for this effect is 0.3-1.5 ng/mL. IGFBP-3 does not inhibit its activity.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE with silver staining.
Host Cell Protein	<0.5 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 100-200 µg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. 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.

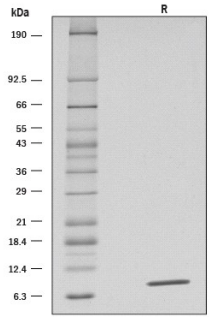
DATA

Bioactivity



GMP-grade Recombinant Human LR3 IGF-I (Catalog # 8335-GMP) stimulates cell proliferation in a serum-free assay using the MCF-7 human breast cancer cell line. The ED_{50} for this effect is 0.3-1.5 ng/mL.

SDS-PAGE



1 µg/lane of GMP-grade Recombinant Human LR3 IGF-I (Catalog # 8335-GMP) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a single band at 9 kDa.

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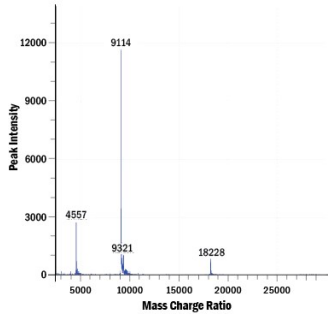
Mass Spectrometry



MALDI-TOF analysis of GMP-grade Recombinant Human LR3 IGF-I (Catalog # 8335-GMP). The major peak at 9114 corresponds to the calculated molecular mass, 9118 Da. The minor peak at 9321 is a matrix-associated artifact of the MALDI-TOF.

BACKGROUND

Insulin-like Growth Factor I (IGF-I), also known as Somatomedin C, is the dominant effector of Growth Hormone (GH) and is structurally homologous to Proinsulin. Human IGF-I is synthesized as two precursor isoforms with N- and alternative C-terminal propeptides (1). These isoforms are differentially expressed by various tissues (1). The 7.6 kDa mature IGF-I is identical between isoforms and is generated by proteolytic removal of the N- and C-terminal regions. Mature human IGF-I shares 94% and 96% amino acid (aa) sequence identity with the mouse and rat orthologs, respectively (2). GH stimulates the production of IGF-I in most tissues (3). Hepatocytes produce circulating IGF-I, while local IGF-I is produced by many other tissues in which it has paracrine effects (1). IGF-I induces the proliferation, migration, and differentiation of a wide variety of cell types during development and postnatally (4, 5). IGF-I regulates glucose, fatty acid, and protein metabolism, steroid hormone activity, and cartilage and bone metabolism (6-11). It plays an important role in muscle regeneration and tumor progression (1, 12, 13). IGF-I binds IGF-I R, IGF-II R, and the Insulin Receptor, although its effects are mediated primarily by IGF-I R (14). IGF-I also binds with strong affinity to IGF binding proteins (IGFBPs), which regulate the availability and biological activities of IGF-I (15, 16).

Long R3 IGF-I (LR3 IGF-I) is a 9.2 kDa synthetic analog of IGF-I that is generated by modifying the aa sequence for mature human IGF-I. These modifications include the substitution of an Arg for Glu at position 3 of the mature IGF-1 sequence and the addition of a thirteen aa N-terminal extension, which is derived from methionyl porcine Growth Hormone (17). These aa changes generate a protein that is still capable of binding to IGF-I and Insulin receptors, but shows considerably lower affinity binding to IGFBPs compared to wild-type IGF-I (17, 18). As a result, LR3 IGF-I has an increased half-life and displays increased biological potency compared to IGF-I (17-22).

References:

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- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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