

Human Insulin-like Growth Factor I (hIGF-I)

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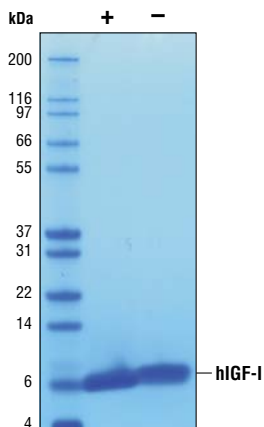
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Source: Recombinant human IGF-I (hIGF-I) Gly49-Ala118 (Accession #P01343) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hIGF-I has a Met on the amino terminus and has a calculated MW of 7,785. DTT-reduced and non-reduced protein migrate as 6 kDa polypeptides. The expected amino-terminal MGPEP of recombinant hIGF-I was verified by amino acid sequencing.

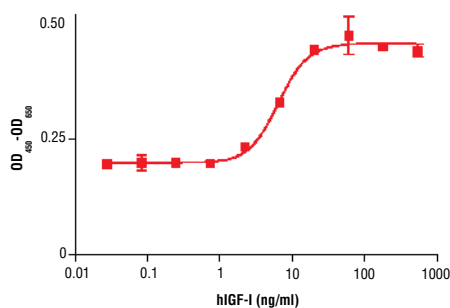
Endotoxin: Less than 0.01 ng endotoxin/1 µg hIGF-I.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hIGF-I. All lots are greater than 98% pure.

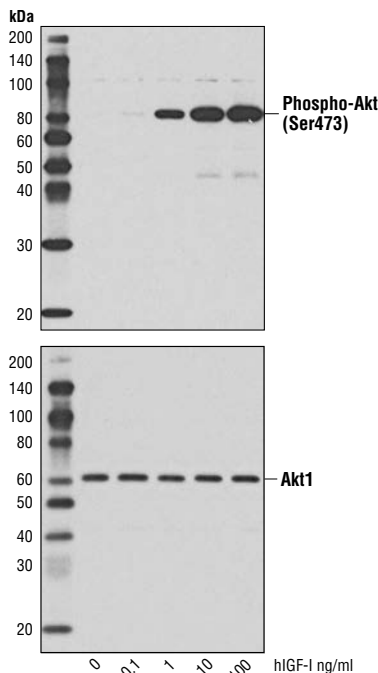


The purity of recombinant hIGF-I was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hIGF-I and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hIGF-I was determined in a cell proliferation assay using primary human dermal fibroblasts. The ED₅₀ of each lot is between 2-8 ng/ml.



◀ The proliferation of primary human dermal fibroblasts treated with increasing concentrations of hIGF-I was assessed. After 72-hour treatment with hIGF-I cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₆₅₀ was determined.



Western blot analysis of extracts from human dermal fibroblasts untreated or treated with hIGF-I for 10 minutes, using Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060 (upper) and Akt1 (C73H10) Rabbit mAb #2938 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl and 20 µg BSA per 1 µg hIGF-I.

Carrier free: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl.

Reconstitution:

With carrier: Add sterile 20 mM citrate, pH 3.0 to a final hIGF-I concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 20 mM citrate, pH 3.0 or 20 mM citrate, pH 3.0 containing protein to minimize absorption of hIGF-I to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hIGF-I should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: Most circulating endocrine acting IGF-I is produced by hepatocytes, and paracrine or autocrine acting IGF-I is produced by defined cell types within specific tissues (1,2). Many neoplastic cells produce IGF-I, which regulates a number of cellular processes including energy metabolism, proliferation, and cell survival (3,4). IGF-I activity is regulated by one or more of the six extracellular IGF-binding proteins (IGFBPs). IGFBPs bind to IGF-I and most inhibit IGF-I binding to IGF-1 receptor (IGFIR) (1,2). Some IGFBPs may increase cell responses to IGF-I. Binding of IGF-I to IGFIR activates the Akt, JNK, and Erk pathways (2). IGF-I and IGFIR are frequently expressed by cancer cells and may contribute to the proliferation and viability of a number of cancer types (1,2).

Background References:

- (1) Pollak, M. (2008) *Nat Rev Cancer* 8, 915-28.
- (2) Chitnis, M.M. et al. (2008) *Clin Cancer Res* 14, 6364-70.
- (3) Karye, K.P. and Sirbasku, D.A. (1988) *Cancer Res* 48, 4083-92.
- (4) Small, T.W. and Pickering, J.G. (2009) *J Biol Chem* 284, 24684-95.