Sterile

#12579

Human $_{His6}$IGFBP3 (h_{His6}IGFBP3)

Cell Signaling TECHNOLOGY®

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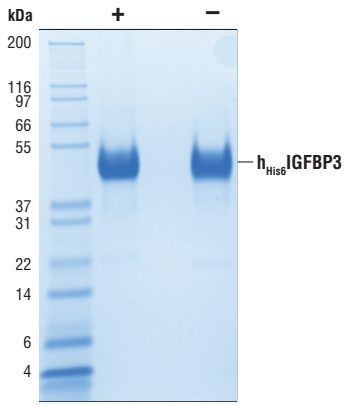
Source: Recombinant Human $_{His6}$IGFBP3 (h_{His6}IGFBP3) Gly28-Lys291 (Accession #NP_17936) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant N-terminally His6-tagged hIGFBP3 has a calculated MW of 30,7123 Da. DTT reduced and nonreduced protein migrate as 50 kDa polypeptides. Lower mobility and heterogeneity in SDS-PAGE are due to glycosylation. The expected amino terminus of recombinant h_{His}6IGFBP3 was verified by amino acid sequencing.

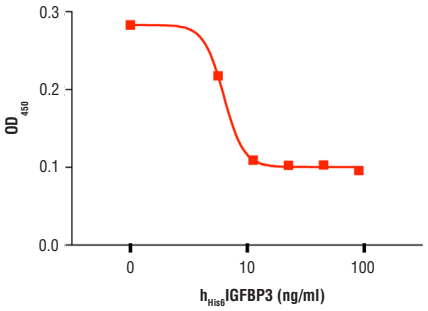
Endotoxin: Less than 0.01 ng endotoxin/1 µg h_{His6}IGFBP3.

Purity: >95% as determined by SDS-PAGE of 6 µg reduced (+) and nonreduced (-) recombinant h_{His6}IGFBP3. All lots are greater than 95% pure.



The purity of recombinant h_{His6} IGFBP3 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant h_{His6} IGFBP3 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of h_{His6}IGFBP3 was determined by inhibition of IGF-I induced Akt phosphorylation in human dermal fibroblasts. The ED_{50} of each lot is between 2.5-9 ng/ml.



The inhibition of IGF-I induced Akt phosphorylation by h_{His6}IGFBP3. Human dermal fibroblasts were treated with Human Insulin-like Growth Factor I (hIGF-I) # 8917 in the presence or absence of increasing concentrations of h_{His6}IGFBP3 for 10 minutes, lysed, and Akt1 (Ser473) phosphorylation was quantified using the PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit #7160.

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of h_{His6}IGFBP3 in 20 mM Tris, pH 7.2 containing 20 µg BSA per 1 µg h_{His6}IGFBP3.

Carrier free: Lyophilized from a 0.22 µm filtered solution of h_{His6}IGFBP3 in 20 mM Tris, pH 7.2.

Reconstitution:
With carrier: Add sterile 20 mM Tris, pH 7.2 or 20 mM Tris, pH 7.2 containing 1% bovine or human serum albumin or 5-10% FBS to a final h_{His6}IGFBP3 concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 20 mM Tris, pH 7.2 or 20 mM Tris, pH 7.2 containing protein to minimize absorption of h_{His6}IGFBP3 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock h_{His6}IGFBP3 should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at 4°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: IGFBP3 is a multifunctional protein that plays a key role in regulation of IGFI/II activity, cell proliferation and death. One of six high-affinity IGF binding proteins, IGFBP3 is the major species in circulation and is bound in a complex with ALS to 99% of hepatic IGF-I (1). Proteolytic degradation of IGFBP3 increases the bioavailability and activity of the IGF I/II (1). However, some biological activities of IGFBP3 are independent of the IGF/IGF-IR axis. IGFBP3 potentiates EGF-induced breast cancer cell proliferation *in vitro* by enhancing ERK phosphorylation and sphingosine kinase-mediated EGFR trans-activation (2,3). Conversely, IGFBP3 has been shown to induce apoptosis and inhibit NF-κB activity (4).

Background References:

(1) Jogie-Brahim, S. et al. (2009) *Endocr Rev* 30, 417-37.

(2) Martin, J.L. et al. (2003) *J Biol Chem* 278, 2969-76.

(3) Martin, J.L. et al. (2009) *J Biol Chem* 284, 25542-52.

(4) Han, J. et al. (2011) *Cancer Lett* 307, 200-10.