SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed IGFBP-3^{HCX}

Source	A DNA sequence encoding the human IGFBP-3 protein sequence (containing the signal peptide sequence, and the mature human IGFBP-3 sequence) was expressed in modified human 293 cells.
Molecular Mass	Symansis IGFBP-3^{HCX} migrates as a broad band between 40 and 45 kDa in SDS-PAGE due to post-translation modifications, in particular glycosylation. This compares with the unmodified IGFBP-3 that has a predicted molecular mass of 28.7 kDa.
pI	Symansis IGFBP-3^{HCX} separates into a number of isoforms with a pI between 6.8 and 8.2 in 2D PAGE due to post-translational modifications, in particular glycosylation. This compares with the unmodified IGFBP-3 that has a predicted pI of 8.76.
% Carbohydrate	Symansis purified IGFBP-3^{HCX} consists of 25-35% carbohydrate by weight.
Purity	>95%, as determined by SDS-PAGE and visualized by silver stain.
Formulation	When reconstituted in 0.5 ml sterile phosphate-buffered saline, the solution will contain 1% human serum albumin (HSA) and 10% trehalose.
Reconstitution	It is recommended that 0.5 ml of sterile phosphate-buffered saline be added to the vial.
Storage	Lyophilized products should be stored at 2 to 8°C. Following reconstitution short-term storage at 4°C is recommended and longer-term storage of aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	The ED50 of IGFBP-3^{HCX} is typically 0.13-0.20 µg/ml as measured by its ability to neutralize rhIGF-II mediated proliferation of the MCF-7 adenocarcinoma cell line.
Background Information	IGFBP-3 is a member of the insulin-like growth factor binding protein (IGFBP) family. This family includes IGFBP-1 to IGFBP-6 that bind to the insulin-like growth factors (IGFs), thus regulating the half-life, activity, transport, and tissue distribution of the IGFs. Structurally, the IGFBP family of proteins is defined by highly homologous amino and carboxy terminal domains with a structurally diverse central region. The IGF binding motifs exist in the amino and carboxy terminal regions. The carboxy terminal domains may also be involved in interactions with numerous other molecules that may modulate IGF-binding as well as conferring IGF-independent function. IGFBP-3 is the most abundant IGFBP found in human serum where it is predominantly found as a ternary complex with IGF-I or IGF-II and the acid-labile subunit (ALS). This complex accounts for the majority of circulating IGF. Functionally, IGFBP-3 has been observed to exhibit both cell growth promoting and inhibiting effects depending on its effect on the bioavailability of IGF, and its IGF-independent function. IGFBP-3 is a 264 amino acid glycoprotein. In addition to glycosylation IGFBP-3 can also be phosphorylated and these post-translational modifications influence functional activity of IGFBP-3 by modulating tissue targeting, cell interaction and susceptibility to proteolytic cleavage. IGFBP-3 contains three potential N-linked glycosylation sites (Asn-X-Ser/Thr) located in the central linker domain at Asn 89 Asn 109, and Asn 172, while phosphorylation occurs at Ser 111 and 113. For a recent review of the IGFBPs please refer to Bach LA (2005) *Trends Endocrinol Metab*. 16(5):228-234 or Firth SM (2002) *Endocr Rev* 23(6):824-854.

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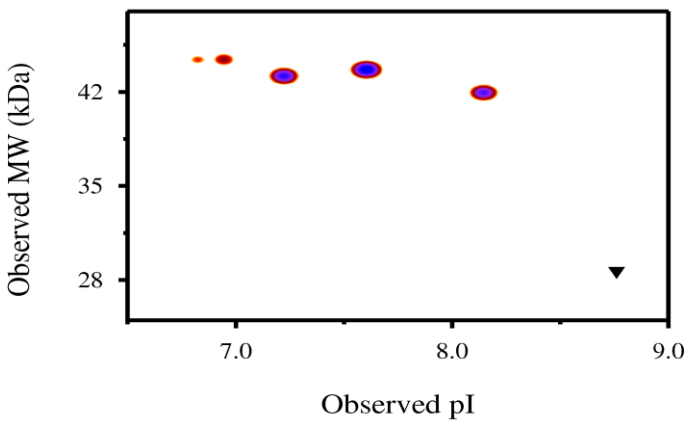
Densitometry

Post-translational modifications result in protein heterogeneity. The densitometry scan demonstrates the purified human cell expressed protein exists in multiple isoforms, which differ according to their level of post-translational modification. Expression of these isoforms is highly significant for cell biology, as they more closely resemble the native human proteins.



The triangle indicates theoretical pI and MW of the protein. The original 2D gel from which the densitometry scan was derived is available upon request.

Theoretical Sequence

GASSGGLGPVVRCEPCDARALAQCAPPPAVCAELVREPGCGCCLTCALSEGQPCGIYTE
RCGSGLRCQPSPDEARPLQALLDGRGLCVNASAVSRLRAYLLPAPPAPGNASESEEDRS
AGSVESPSVSSTHRVSDPKFHPLHSKIIIIKKGHAKDSQRYKVDYESQSTDTQNFSSESKR
ETEYGPCRREMEDTLNHLKFLNVLSPRGVHIPNCDKKGFYKKKQCRPSKGRKRGFCWCV
DKYGQPLPGYTTKGKEDVHCYSMQSK