

Technical Bulletin

The Science of LONG[®]R³IGF-I Features and Benefits

LONG R³IGF-I: Manufactured Exclusively for Cell Culture Use

LONG R³IGF-I is a recombinant growth factor analog of human insulin-like growth factor I (IGF-I). It is specifically designed and manufactured for mammalian cell culture and is intended to support large-scale manufacturing of biopharmaceuticals. LONG R³IGF-I is currently used in several biopharmaceuticals approved by the FDA (United States), EMEA (Europe) and MHLW (Japan). When supplemented in serum-free cell cultures LONG R³IGF-I binds specifically to type I IGF receptors (IGF-IR) and promotes cell proliferation, increased cell survival and increased productivity through greater proliferate and anti-apoptotic signalling. LONG R³IGF-I provides equivalent or better performance to recombinant insulin depending on the cell line and clone. (See figures 1, 2, 3.)

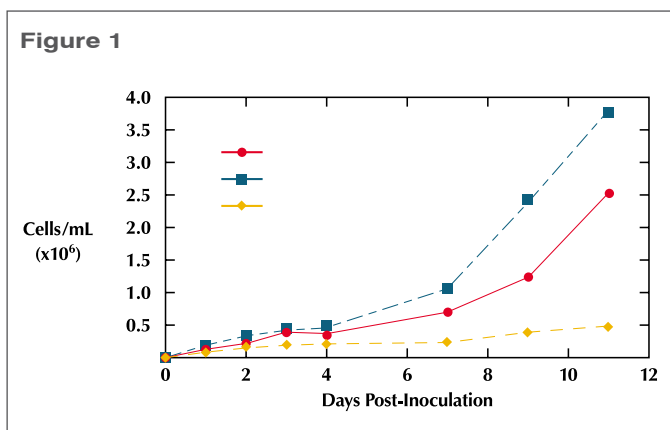


Figure 1: CHO cell number in serum-free media containing LONG R³IGF-I or insulin

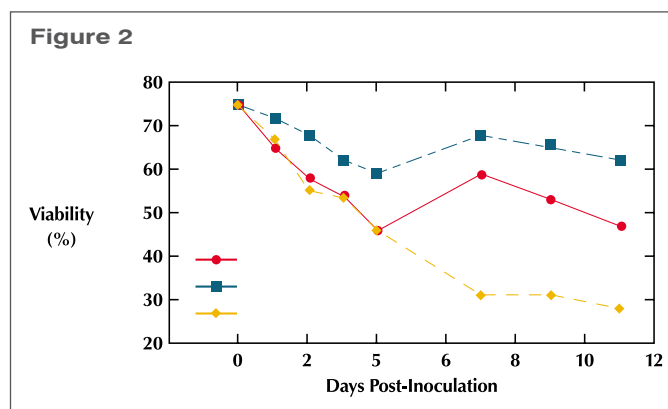


Figure 2: Viability of CHO cells in serum-free media containing LONG R³IGF-I or insulin

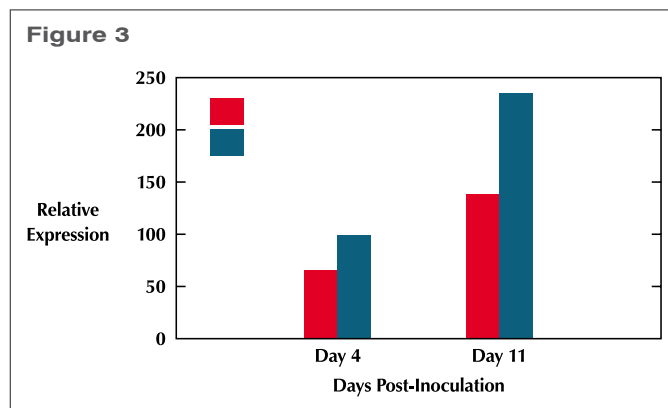


Figure 3: Cumulative Recombinant Protein Production in CHO Cells

LONG R³IGF-I also has many industrial and academic research applications. The compound has shown utility in the study of IGF-I ligand-receptor binding kinetics, receptor regulation, IGF/insulin receptor cross-talk and the interactions of IGF-I binding proteins (IGFBP's) with

IGF-I and the IGF-IR⁶. LONG^{R3}IGF-I is also useful for elucidating the intracellular signaling pathways involved with mitogenesis, cell proliferation and apoptosis¹. All cells that have a growth response to insulin in cell culture have the potential to respond to LONG^{R3}IGF-I. LONG^{R3}IGF-I is effective in commercially relevant cell types including CHO, PER.C6[®] and HEK 293. Hybridomas and fibroblasts have also been shown to respond to LONG^{R3}IGF-I.

Better Science for Better Cell Culture

LONG^{R3}IGF-I is an analog of human insulin-like growth factor I (IGF-I) specifically engineered by Novozymes Biopharma for use in industrial cell culture. Insulin and IGF-I and their receptors — the insulin receptor (IR) and the type-I receptor (IGF-IR) — have similar amino acid sequence and protein structure. As a consequence, insulin and IGF-I are able to bind to each other's receptor with relatively low affinity. It is widely accepted that in CHO cells the effects of insulin are mediated by the IGF-IR, due to the fact there are relatively few IR present on CHO cells and that insulin must be present at a high, non-physiological concentration, typically 1 - 10 mg/L, to be effective. A more effective growth factor is one which targets and activates the IGF-IR directly, such as IGF-I or the analog LONG^{R3}IGF-I. LONG^{R3}IGF-I has a distinct biological advantage over native IGF-I due to its low affinity for IGF Binding Proteins (IGFBPs). All mammalian cells secrete IGFBPs which bind to

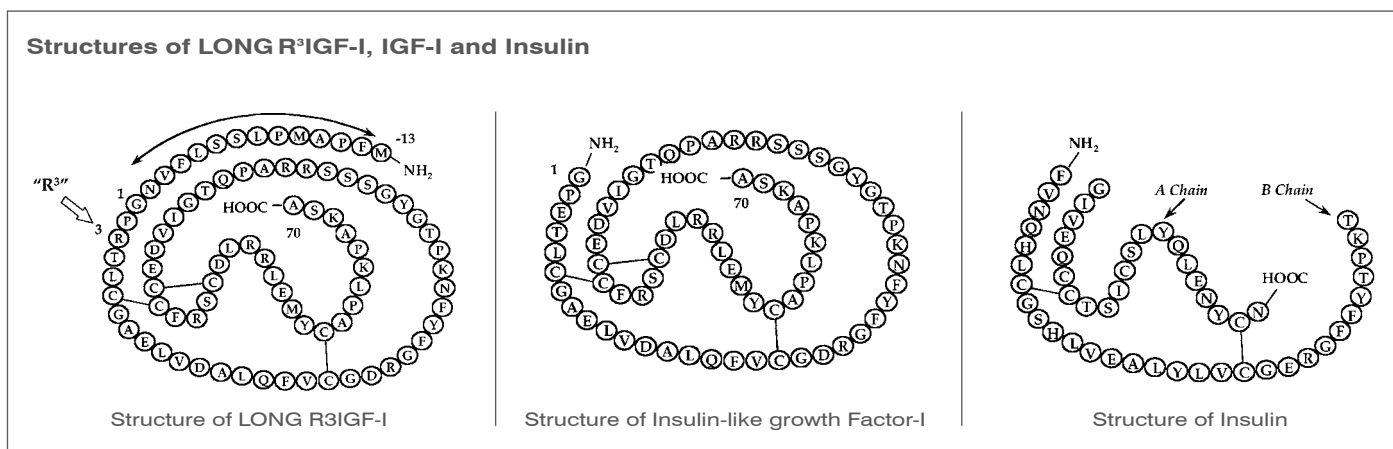
and inhibit native IGF-I. The substitution of an arginine for glutamic acid at position 3 in LONG^{R3}IGF-I, in conjunction with the 13 amino acid N-terminal extension peptide, results in >1000-fold reduced affinity for IGFBPs enhancing its bioavailability and effectiveness in comparison to native IGF-I.

Increase Cell Density, Maintain Higher Viability and Extend Culture Duration

Under bioreactor conditions, stress-induced apoptosis is the major cause of loss of cell viability. Activation of the IGF-IR results in the stimulation of a number of signal transduction cascades that have been identified as important for cell survival and proliferation. LONG^{R3}IGF-I only results in greater activation of the IGF-IR over insulin, but also results in greater activation of key anti-apoptotic and proliferative signalling molecules.

Prolonged Cell Culture Activity through Greater Stability

Under normal cell culture conditions, LONG^{R3}IGF-I is more stable than insulin, persisting up to 2 times longer. Insulin is degraded in cell culture by enzymes (insulinases) secreted by cells into culture media. In addition, insulin is more rapidly internalized and degraded compared with IGF-I and LONG^{R3}IGF-I. The extended culture stability of LONG^{R3}IGF-I results in prolonged activity and associated benefits to cell culture.



User-friendly Formulations for Greater Flexibility

Unlike recombinant insulin and other growth factors, LONG^RIGF-I is available as either a lyophilized powder (Catalog No. 85580C) or a liquid formulation (Catalog No. 91590C). Both formats are stable at 2 to 8 C. The liquid formulation is ready to use — there is no need to thaw or reconstitute; just open and dilute directly into cell culture media. LONG^RIGF-I can also be milled into media.

In Summary

- LONG^RIGF-I is manufactured exclusively for cell culture applications.
- LONG^RIGF-I binds with high affinity to IGF-I receptors and in many cell types potently stimulates proliferation and increases culture viability and specific recombinant protein production.
- LONG^RIGF-I binds with very low affinity to IGF-binding proteins, making it more biologically active than native IGF and allowing easier study of the IGF-I receptor and its actions.
- LONG^RIGF-I has also been shown to stimulate anti-apoptotic pathways, promote cell survival and aid transition to serum-free media.

Comparative Biology of Insulin, IGF-I and LONG ^R IGF-I			
Property	Insulin	IGF-I	LONG ^R IGF-I
Potency in Cell Culture	Low (mg/L)	High (µg/L)	High (µg/L)
Receptor	Insulin Receptor: 2 α and β chains Tyrosine kinase	Type I IGF receptor: 2 α and β chains Tyrosine kinase	Type I IGF receptor: 2 α and β chains Tyrosine kinase
Binding to type I IGF receptor	Low affinity	High affinity	High affinity
Binding to insulin receptor	High affinity	Low affinity	Low affinity
Binding to IGF binding proteins	No Binding	High affinity	No Binding
Structure	Heterodimer	Monomer	Monomer
Size	51 amino acids 6 kDa	70 amino acids 7.4 kDa	83 amino acids 9.11 kDa
Stability in cell culture	Relatively unstable	Stable	Stable

Test	Specification
Appearance	Lyophilized white/creamy powder or a clear liquid
Biological Activity	ED50 < 10 ng/mL (stimulation of protein synthesis in L6 myoblasts)
Endotoxin	< 0.10 EU/µg protein
Identity	Confirmed by N-terminal sequence analysis and HPLC (18 residues > 95% single sequence)
Molecular Weight	9108 9112 daltons
Purity	≥ 95% as determined by SDS-PAGE
Catalog Number	Available Sizes
85580C	5, 20 and 50 mg
91590C	5 mL, 100 mL

References

1. Yandell, C., Lawson, J., Butler, I., Wade, B., Sheehan, A., Grosvenor, S., Goddard, C. and Simula, T., An Analog of IGF-I, A Potent Substitute for Insulin in Serum-Free Manufacture of Biologics by CHO Cells, Bioprocess International, March 2004.
2. Francis, G.L., Ross, M., Ballard, F.J., Milner, S.J., Senn, C., McNeil, K.A., Wallace, J.C., King, R., and Wells, J.R., Novel Recombinant fusion protein analogues of insulin-like growth factor (IGF)-I indicate the relative importance of IGF-binding protein and receptor binding for enhanced biological potency, *J. Mol. Endocrinol.* 1992, Jun; 8(3): 213-23.
3. Morris, A.E. and Schmid, J., Effects of Insulin and LONG™R³ on Serum-Free Chinese Hamster Ovary Cell Cultures Expressing Two Recombinant Proteins, *Biotechnol. Prog.* 2000, 16: 693-697.
4. Chun, C., Heineken, K., Szeto, D., Ryll, T, Chamow, S. and Chung, J.D., Application of Factorial Design to Accelerate Identification of CHO Growth Factor Requirements, *Biotechnol. Prog.* 2003, 19: 52-57.
5. Thomas, J.N. and Fung, V., Comparison of LONG™R³IGF-I with insulin in the support of cell growth and recombinant protein expression in CHO cells, In *Animal Cell Technology, Products of Today, Prospects for Tomorrow*. European Society for Animal Cell Technology. The 12th Meeting. Ed.'s Spier, R.E., Griffiths, J.B., & Berthold, W. Butterworth-Heinemann UK, 1994.
6. Pampusch, MS, Xi, G, Kamanga-Sollo, E, Loseth, KJ, Hathaway, MR, Dayton, WR and White, ME. Production of recombinant IGF-binding protein-5 and its effect on proliferation of porcine embryonic myoblast cultures in the presence and absence of IGF-I and LONG™R³IGF-I, In *J Endocrinol.* 2005 Apr;185(1):197-206.
7. Drapeau, D., Luan, Y., Popoloski, J.A., Richards, D.T., Cohen, D.C., Sinacore, M.S. and Adamson, S.R., Extracellular insulin degrading activity creates instability in a CHO-based batch-refeed continuous process, *Cytotechnology* 1994, 15:103-109.
8. Zapf, A., Hsu, D. and Olefsky, J., Comparison of the Intracellular Itineraries of Insulin-Like Growth Factor-I and Insulin and Their Receptors in Rat-I Fibroblasts, *Endocrinology*, 1994, 134(6): 2445-52.

Warranty, Limitation of Remedies

SAFC Biosciences warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC Biosciences' written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC Biosciences be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC Biosciences expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

SAFC Biosciences is a registered trademark of Sigma-Aldrich Biotechnology L.P and Sigma-Aldrich Co. LONG is a registered trademark of Novozymes Biopharma AU. Per.C6 is a registered trademark of Crucell N.V.

© 2010 SAFC Biosciences, Inc.

Issued June 2010 T085
0508 0510