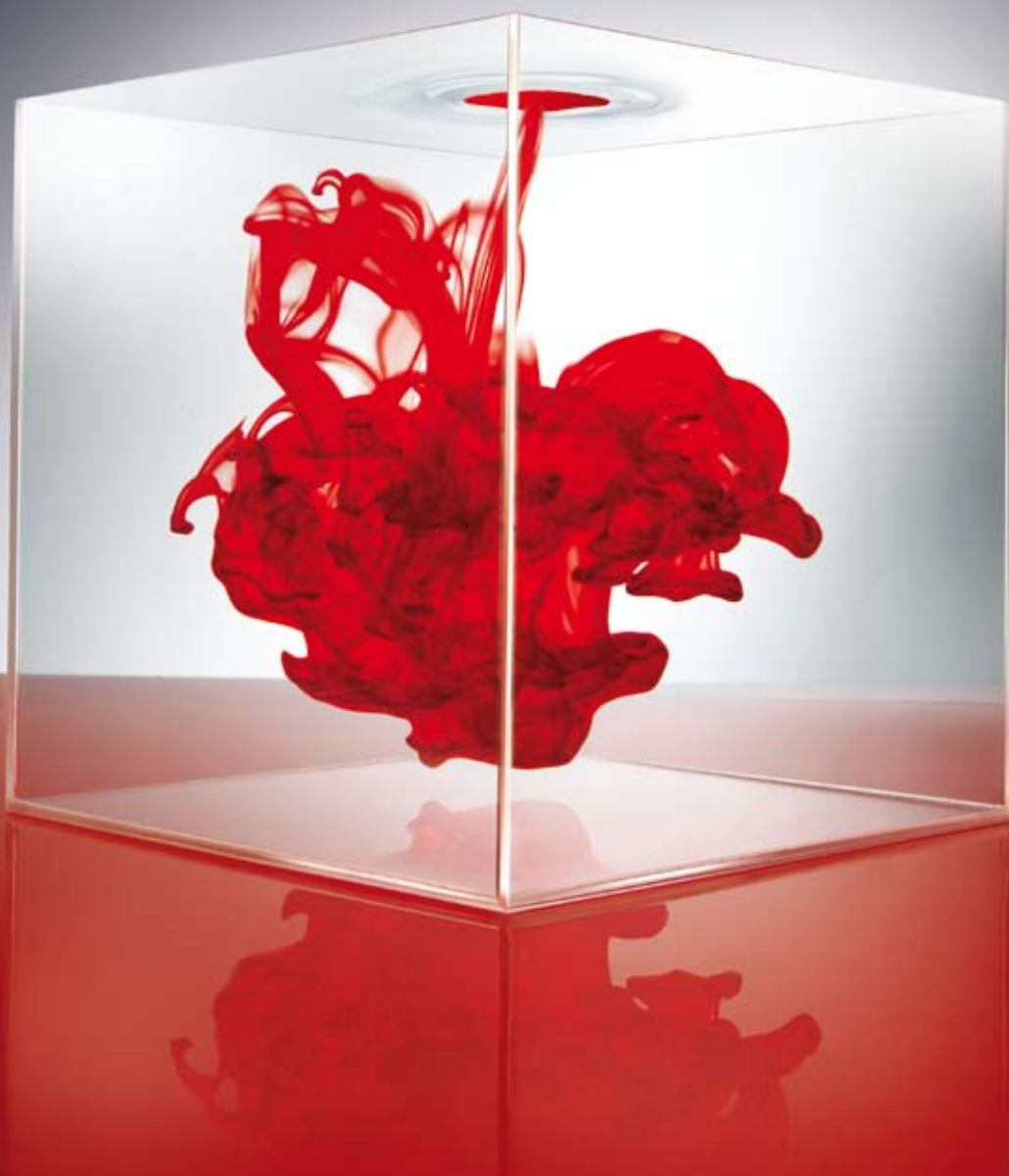


LONG[®]R³IGF-I

Your insulin alternative



SAFC Biosciences™
| Accelerate Success™

safcglobal.com

SIGMA-ALDRICH®

LONG[®]R³IGF-I

Your insulin alternative

A growth factor manufactured exclusively for industrial cell culture

Recombinant human insulin and other growth factors are essential for long-term growth and proliferation of cell lines. Although insulin is used as a growth factor in cell culture, its primary use is as a therapeutic drug for the treatment of diabetes. This has led to supply and availability issues for industrial cell culture users. In contrast, LONG[®]R³IGF-I is a dedicated raw material manufactured exclusively for cell culture applications providing a consistent, compliant and reliable alternative to recombinant insulin. It is not subject to market fluctuations and shortages as is recombinant insulin.

Proven science to maximize

cell culture performance

When LONG[®]R³IGF-I is supplemented into serum-free media it promotes cell proliferation, increased cell survival, increased productivity through greater proliferation and anti-apoptotic signaling. LONG[®]R³IGF-I provides equivalent or better performance to recombinant insulin depending on the cell line and clone.

LONG[®]R³IGF-I has been used successfully with numerous cell types including CHO, BHK, HEK 293, Vero, PER.C6[®], MDCK and fibroblasts (graphs 1-4 page 4). All cell types that have a growth response to insulin in cell culture have the potential to respond to LONG[®]R³IGF-I.

Independent analysis of LONG[®]R³IGF-I in cell culture

Company	Benefit	Reference
Immunex	LONG [®] R ³ IGF-I resulted in higher CHO cell growth and viability.	Morris <i>et al</i> , Biotechnology Progress, 2000, 16, 693-697.
Abgenix	LONG [®] R ³ IGF-I resulted in higher CHO cell growth, viability and eased adaptation to serum-free media.	Chun <i>et al</i> , Biotechnology Progress, 2003, 19, 52-57.
Seattle Genetics	LONG [®] R ³ IGF-I resulted in maintaining higher CHO cell viability and extended culture duration.	Sundra <i>et al</i> , Seattle Genetics, Cell Culture Engineering 10th annual meeting.
University of Birmingham, UK	LONG [®] R ³ IGF-I resulted in higher HEK 293 growth and maintained higher culture viability.	Buckler <i>et al</i> , University of Birmingham, JAACT 19th annual meeting.

LONG®R³IGF-I is a highly potent and effective alternative to recombinant insulin for cell culture applications. It has been engineered specifically for supplementation of serum-free cell culture media to enhance the survival and proliferation of mammalian cells.

LONG®R³IGF-I is not subject to market fluctuations and shortages as is recombinant insulin.



Benefit from the proven alternative

Better science for better cell culture

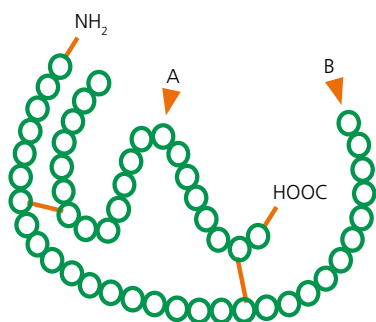
LONG[®]R³IGF-I is an analogue of human-like growth factor I (IGF-I) specifically engineered for use in industrial cell culture. Insulin, like IGF-I and their receptors (the insulin receptor (IR) and the type-I IGF receptor IGF-IR) have a similar amino acid sequence and protein structure. As a consequence, 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 the 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

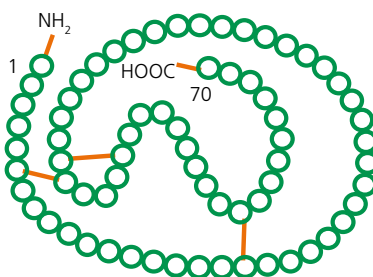
which targets and activates the IGF-IR directly, such as IGF-I or the analogue LONG[®]R³IGF-I.

LONG[®]R³IGF-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 glutamine acid at position three in LONG[®]R³IGF-I, in conjunction with the 13 amino acid N-terminal extension peptide, results in > 1000-fold reduced affinity for IGFBPs enhancing bioavailability and effectiveness in comparison to native IGF-I.

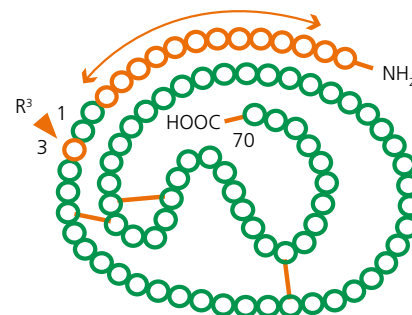
Structures of LONG[®]R³IGF-I, IGF-I and Insulin



Insulin

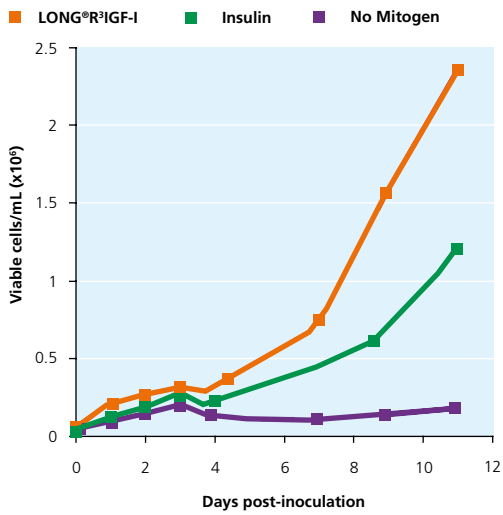


Native IGF-I

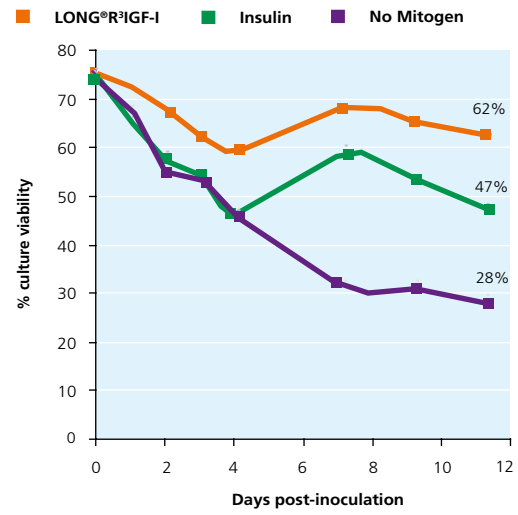


LONG[®]R³IGF-I

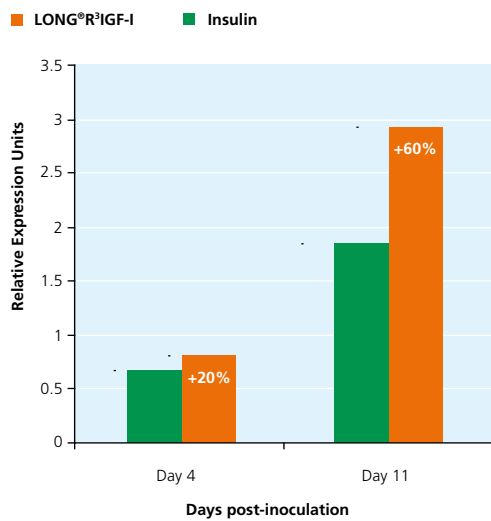
Graph 1 CHO cell growth



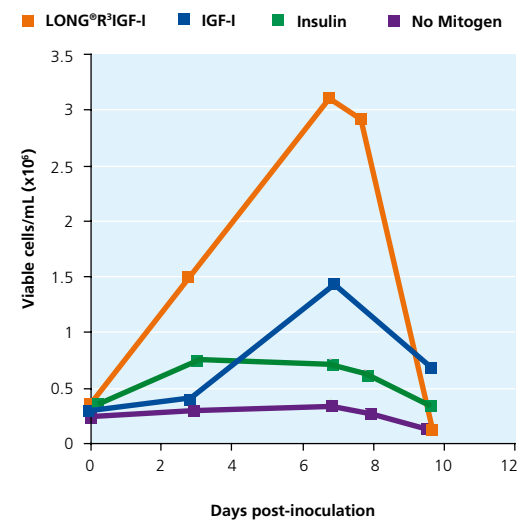
Graph 2 CHO cell viability



Graph 3 CHO productivity



Graph 4 HEK 293 cell growth



Graph 1-3: Growth, viability and production data for insulin versus LONG^RIGF-I. CHOK1 cells producing a recombinant protein were adapted to growth in protein-free media. Cells were cultured in spinner flasks in serum-free medium (60 mL) containing either insulin at 10 mg/L, LONG^RIGF-I at 50 μ g/L or no growth factor for a period of 11 days in a modified fed-batch process.

Graph 4: Growth data for insulin, native IGF-I and LONG^RIGF-I. HEK 293 cells were adapted to growth in a protein-free media. Cells were cultured in 250 mL shaker flasks in serum-free medium (60 mL) containing either native IGF-I at 100 μ g/L, insulin at 1 mg/L, LONG^RIGF-I at 100 μ g/L or no growth factor for a period of 10 days in a batch process.



Enhance your cell culture capabilities

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®R³IGF-I not only results in greater activation of IGF-IR over insulin, but also results in greater activation of key anti-apoptotic and proliferative signaling molecules: Akt and MAPK. Increased activation of these signaling molecules prevents the loss of viability caused by different culture conditions (graphs 5-8 opposite page).

Prolonged cell culture activity through greater stability

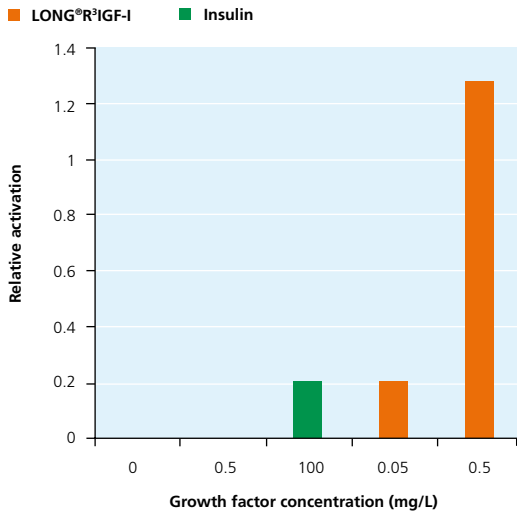
Under normal cell culture conditions, LONG®R³IGF-I is more stable than insulin, persisting up to 2 times longer. Insulin is degraded in cell culture by enzymes (insulinases) secreted by cells into culture media. In addition, insulin is more rapidly internalized and degraded compared with IGF-I and LONG®R³IGF-I. The extended cell culture stability of LONG®R³IGF-I results in prolonged activity and associated benefits to cell culture. (graph 9 opposite page).

User-friendly formulations for greater flexibility

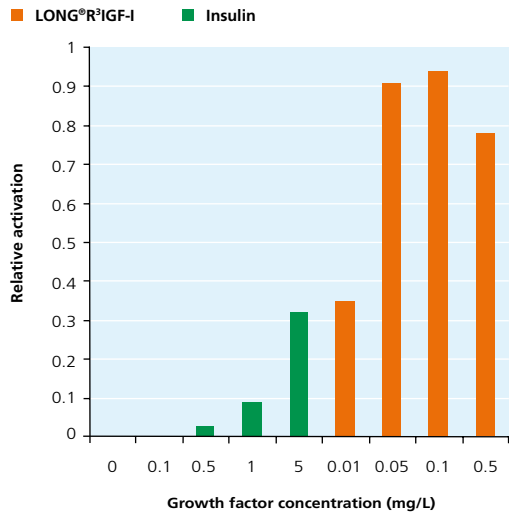
Unlike recombinant insulin and other growth factors, LONG®R³IGF-I is available as either a lyophilized powder (catalog no. 85580C) or liquid formulation (catalog no. 91590C). Both formats are stable at 2 to 8°C. The liquid formulation is ready to use, there is no need to thaw or reconstitute, just open and dilute directly into cell culture media. LONG®R³IGF-I can also be milled into your media and feed formulations.



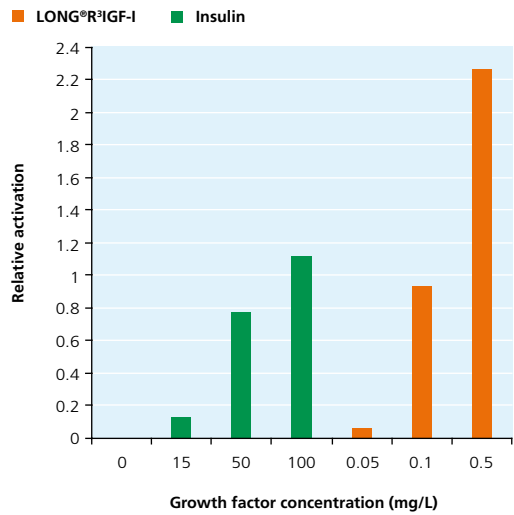
Graph 5 Activation of IGF-IR in CHO cells



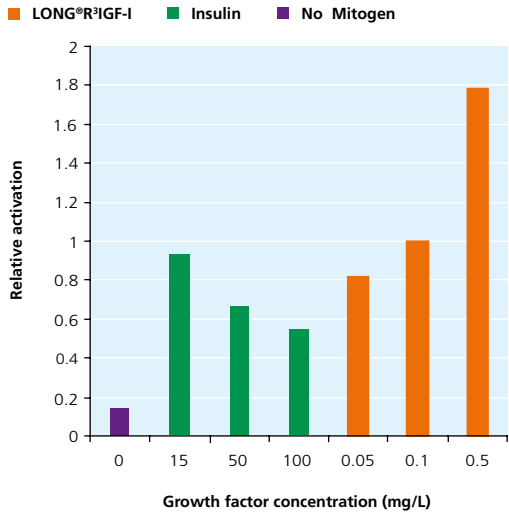
Graph 6 Activation of IGF-IR in HEK 293 cells



Graph 7 Activation of AKT in CHO cells

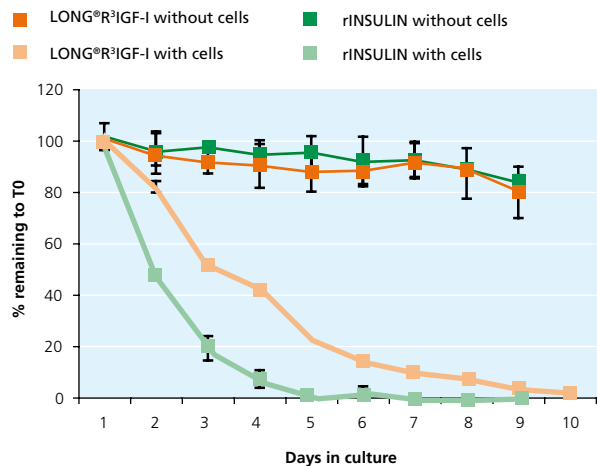


Graph 8 Activation of MAPK in CHO cells



Graph 5-8: Activation of IGF-IR, mitogenic and anti-apoptotic signal molecules were detected by measuring the level of phosphorylation by immunoblotting with specific anti-phospho antibody. Relative activation for IGF-IR is expressed as a ratio of phosphorylated receptor to unphosphorylated receptor. Relative activation of MAPK and Akt is expressed as a ratio of phosphorylated protein to total protein (β actin).

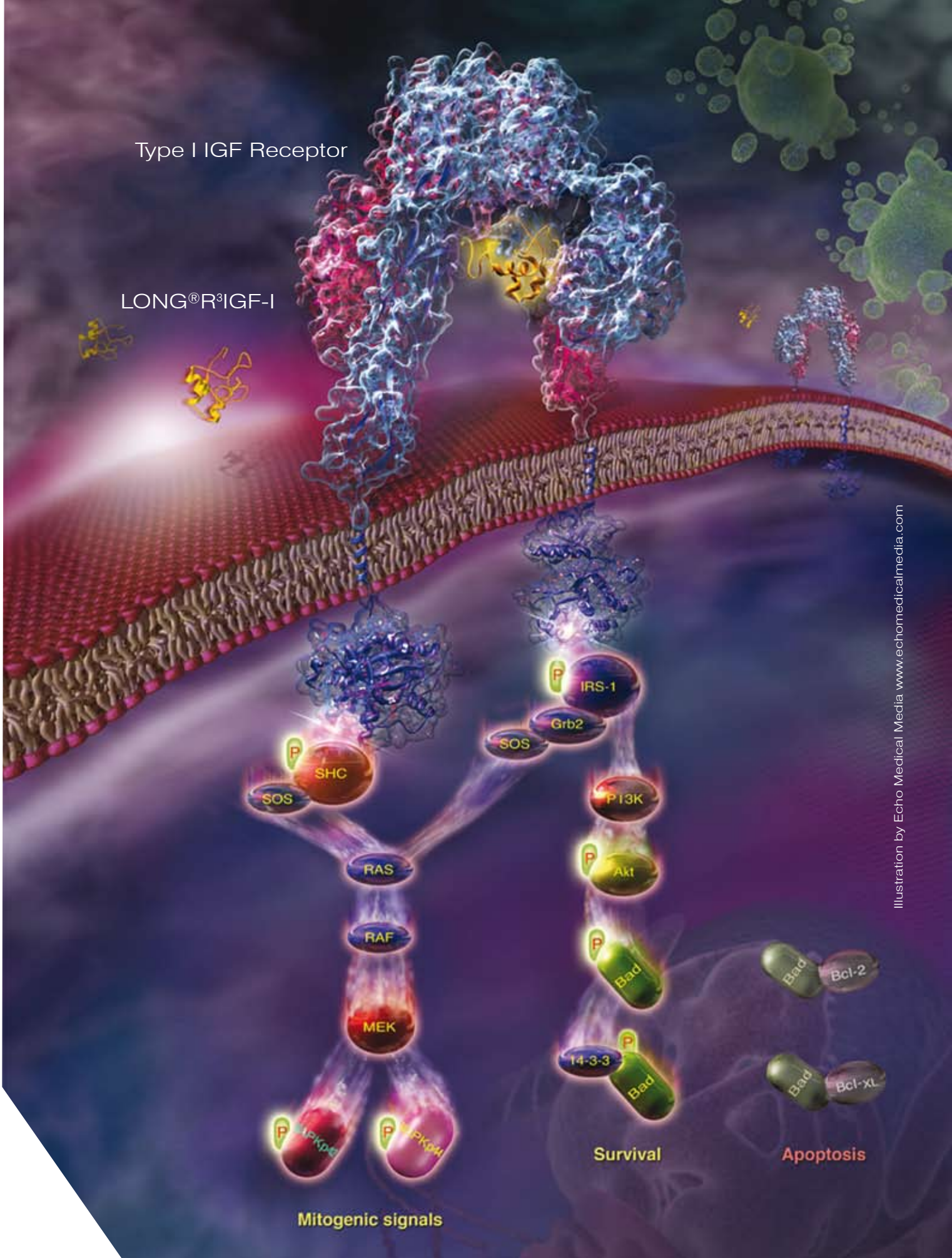
Graph 9 Cell culture stability data for insulin versus LONG®R³IGF-I



LONG®R³IGF-I resulted in increased cell growth, viability and productivity (data not shown).

Type I IGF Receptor

LONG[®]R³IGF-I



A consistent and compliant solution


Regulatory compliant cGMP and animal free

LONG®R³IGF-I is manufactured under cGMP compliance with the International Conference on Harmonization (ICH) Q7A guidelines. The manufacturing plant is regularly audited by major European, US and Japanese biopharmaceutical companies. LONG®R³IGF-I is produced in a proprietary *E. coli* fermentation process that is completely animal free (AF). No animal-derived materials are used during the manufacture or storage of LONG®R³IGF-I. A certificate of origin is available upon request.

Manufactured by Novozymes Biopharma, LONG®R³IGF-I has been supplied to the industrial cell culture market for over 15 years. In addition, LONG®R³IGF-I is used in the manufacture of several FDA (US), EMEA (Europe) and MHLW (Japan) approved marketed products, with many more in late-phase clinical trials.

Test	Specification
Appearance	Lyophilized white/creamy crystalline powder or a clear liquid
Endotoxin	< 0.10 EU/ μ g protein
Bioburden	Total viable aerobic count \leq 100 cfu/mL
Biological Activity	ED ₅₀ < 10 ng/mL (Bioassay assessing the stimulation of protein synthesis in L6 myoblasts)
Concentration	0.9-1.1 mg/mL by reverse-phase HPLC
Identity	Confirmed by N-terminal sequence analysis and reverse-phase HPLC (18 residues > 95% single sequence)
Purity	A single band \geq 95% as determined by SDS-PAGE





**Proven science to help
maximize your cell
culture performance**

- Promotes cell proliferation
- Increases cell survival
- Activates proliferative and anti-apoptotic signaling molecules
- Extends culture longevity
- Eases transition to serum-free media
- Increases recombinant protein production
- Up to 200 times more potent than insulin
- Greater cell culture stability

For more information on the LONG[®]R³IGF-I product line, please visit

www.safcglobal.com

Ensure supply security and total reliability

All backed by a comprehensive risk management strategy

SAFC Biosciences is committed to protecting our customers by providing accurate forecasts to Novozymes, our manufacturing partner, necessary to meet supply demands. As a result, the supply of LONG®R³IGF-I is not capacity constrained. Currently, Novozymes has the capacity to produce enough LONG®R³IGF-I to supplement up to 160 million liters of serum-free media annually and can easily be expanded.

LONG®R³IGF-I production and supply is backed by a comprehensive risk management strategy, which includes significant inventory build-up, offsite secure storage facilities and second site manufacturing contingencies.

SAFC Biosciences Responsive Expertise

SAFC Biosciences is the world's leading supplier of cell culture reagents to producers of marketed biological products. We are committed to supplying high-quality critical raw materials and specialized cell culture products to the global health care industry. By providing the broadest range of highly customized products and services possible to cell culture-based manufacturing companies, we demonstrate our commitment to being responsive experts.

Dedicated SAFC Biosciences™ Technical Managers are available to guide you through your evaluation and ongoing use of LONG®R³IGF-I.

LONG®R³IGF-I Liquid, 5 mL	91590C-5ML	5 mL Type 1, glass vial with crimp seal
LONG®R³IGF-I Liquid, 100 mL	91590C-100ML	125 mL Nalgene® PET bottle

LONG®R³IGF-I lyophilized powder, 1 mg	85580C-1MG
LONG®R³IGF-I lyophilized powder, 5 mg	85580C-5MG
LONG®R³IGF-I lyophilized powder, 10 mg	85580C-10MG
LONG®R³IGF-I lyophilized powder, 20 mg	85580C-20MG
LONG®R³IGF-I lyophilized powder, 50 mg	85580C-50MG

Validated material is available with minimum specified balances maintained in SAFC Biosciences inventory.



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Accelerate Success™