

Pilot Study Considerations

What makes Short™ AE-IGF-1 different from other competing IGF-1 molecules?

Short™ AE-IGF-1 differs from human IGF-1 by two additional amino acids linked to the molecule's N-terminus. This proprietary extended molecule was **explicitly designed for cell culture biomanufacturing to achieve two main benefits:**

- **Increased expression, with better yields** in refolding and recovery steps during production of Short™ AE-IGF-1;
- **Enhanced stability** of Short™ AE-IGF-1 within bioproduction processes and **extending specific activity** per unit throughout those processes.

The proprietary nature of the molecule and our manufacturing processes allow us to offer superior performance, a better quality product, at a significant reduction in cost while ensuring a high level of the specific activity of the growth factor in increasing biopharmaceutical yields.

Short™ AE-IGF-1, being highly biologically potent, significantly increases recombinant protein production while reducing apoptosis. This premium bioactive grade growth factor is certified 100% animal-free and ideal for both research and large-scale culture systems and bioreactors.

Confidence in compliance and security of supply?

Short™ AE-IGF-1 is produced and tested under ISO 18001, ISO 9001, ISO 14001 certified conditions and strict FDA, EMA, and GMP guidelines. Our comprehensive supply and risk management strategy includes an inventory surplus equal to 2 years of forecast demand maintained at secure storage facilities.

What product support does CellRx provide?

All cells with a functional Type 1 IGF receptor will respond to Short™ AE-IGF-1, including commercially available cell lines such as CHO, PER.C6, BHK, HEK 293, embryonic stem cells, mesenchymal stem cells, hematopoietic stem cells, fibroblasts, and hybridomas.

To increase an individual's cell culture's specific performance, CellRx can help the **setup and optimisation of your culture conditions**. Drawing on more than thirty years of cell culture development expertise, our specialists are on hand to support your program's optimisation. We look forward to hearing from you.

What are the typical initial seeding densities for cell culture optimisation using Short™ AE-IGF-1 with mammalian cells?

The Customer should note that seeding densities vary depending on the mammalian cell line under consideration and the specific bioreactor system. In general:

- Initial seeding densities range, at the time of inoculation, from 2×10^5 to 1×10^6 viable cells/ml in a fed-batch system;
- Daily feeding strategy between 2 to 12 days, with 6 to 12 days achieving the highest cell mass levels.

What optimum concentration of Short™ AE-IGF-1 does CellRx recommend for cell culture optimisation?

The customer should note that the optimum concentration that gives maximum yield is very dependent on the cell line and the nature of its genetic modification to produce a recombinant product. Conditions similar to those used for other IGF-1 molecules can be replicated for Short™ AE-IGF.

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Based on collated information, CellRx recommendations are:

- For optimisation studies in **Batch culture systems**, we recommend a testing range between 25-200ug/L (25-200ng/ml). CellRx advises using sterile 100 mM Acetic Acid (pH 2-4) to make any required concentration adjustment.
- In general, the best expression yields of the recombinant product in Batch culture observed with Short™AE-IGF-1 is at a concentration range of 50-100ug/L (50-100 ng/ml). In some studies performed at concentrations of 150 ug/L to 200 ug/L, recombinant product yields remained constant.
- In **fed-batch systems**, a concentration of 150 ug/L (150 ng/ml) is used, allowing greater flexibility in optimising the FEED-RATE. As a guide, if a Customer is using an existing IGF-1 molecule, we recommend starting your optimisation with the Short™AE-IGF at a plus/minus 25-30% concentration.
- In **perfusion systems**, the optimum range has consistently been between 25-100 ug/L (25-100 ng/ml). Predicating the particular cell line/recombinant protein test system combination under optimisation, we have seen that lower concentrations achieved the highest levels of protein production (note: these are guide ranges, optimum concentration has to be experimentally determined). As with all perfusion systems, the Short™AE-IGF concentration remained constant, especially as cell mass achieves its maximum level around day 6-12.

Does CellRx have clearance data on Short™AE-IGF-1 to demonstrate it is used up in downstream bioprocessing?

Short™AE-IGF has a **similar enzyme degradation profile** to other IGF-1 growth factors. Any assay established for IGF-1 will be almost identical for Short™AE-IGF.

Do you have supporting data on TSE/BSE statements, MSDS, C of A?

Yes. Short™AE-IGF-1 is manufactured in a proprietary *E. coli* fermentation process and conforms to all **FDA, QSR guidelines**, is certified **100% animal-free** to a tertiary level, and has requisite Certificate of Analysis.

CellRx also has ISO 18001, ISO 9001, ISO 14001 documentation to satisfy external audit teams relating to the CellRx manufacturing facility in Pretoria, South Africa.

CellRx, on request, are happy to share copies of the following documents:

- Product Specification
- TSE/BSE Statement
- MSDS
- Animal-Free Production Process
- Certificate of Analysis

What strategies does CellRx use to ensure the security of supply for Short™AE-IGF-1?

CellRx manufactures Short™AE-IGF with a **comprehensive risk mitigation strategy** appropriate for ensuring the security of supply of this critical cell culture raw material.

We manage a **global inventory safety stock** of over two years of forecasted demand, with a current manufacturing capacity exceeding Kg quantity annually.

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Furthermore, CellRx can manufacture a preferred batch size tailored for a particular customer and hold this stock against an agreed monthly/ quarterly/ bi-annual/ annual offtake amount that suits the needs of the customer. This inventory and custom manufacturing options, together with a strategy to transfer our entire manufacturing process to an alternate CMO site within 90 days, using our highly qualified process scientists in the event of a catastrophe, results in our customers experiencing a continuous supply of Short™ AE-IGF.

Are there additional R&D benefits for Customers?

Customers have immediate access to a proprietary *E.coli* expression platform and a growing pipeline of recombinant protein products, including Short™ AE-IGF and IL2. The operation is reliable, scalable, and flexible to meet the increasing needs of our customers

CellRx's proprietary bioproduction process includes:

- Development of a hyper-production *E.coli* clone.

- Implementation of a Process Flow to allow both cost-competitive and maximum production efficiency.
- Development and Implementation of flexible production workflows that will enable processing various batch sizes (USP and DSP can function independently).
- DSP unit operations optimised to ensure limited process losses across all operations.
- Purification unit operation standardised and optimised to maximise recovery and purity yields above 95%.
- Taken in aggregate, this in-house technology and know-how improve overall COGS and manufacturability. These improvements have underpinned the company's ability to meet the security of our Customers' supply needs over any reasonable time frame or offtake.

Further product information and details on custom services are also available on our website at <http://www.cellrxbio.com>

CellRx Short™ AE-IGF-1 – Pilot Study Considerations | Version: 0920-1

Short™ AE-IGF-1 is a registered trademark of CellRx Limited (Synonyms include AE-IGF-1, RhShort AE-IGF-1, Short IGF), and is covered by the following patents assigned to CellRx: US Patents 5,459,052, 5,691,168 and 5,708,134.

CellRx Short AE-IGF-1 is available for laboratory research and large-scale in-vitro biopharmaceutical manufacturing use only. Not for diagnostic or therapeutic use.