

# Product Information

## LONG®R³IGF-I Lyophilized, Media Grade

CATALOG NO. 85580C

### Description

LONG®R³IGF-I is a recombinant analog of human insulin-like growth factor-I (IGF-I) that has been specifically engineered for the enhancement of cell culture performance. LONG®R³IGF-I is more biologically potent *in vitro* than either insulin or native IGF-I and has been shown to significantly increase recombinant protein production. It is ideal for both research and large-scale culture systems utilizing serum-free or low-level serum applications. All cells that have a growth response to insulin in cell culture have the potential to respond to LONG®R³IGF-I. LONG®R³IGF-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®R³IGF-I. LONG®R³IGF-I is produced in a patented *E. coli* expression system without the use of animal-derived components.

LONG®R³IGF-I is also available as a 1 mg/mL liquid formulation in 100 mM acetic acid, Catalog No. 91590C.

### Precautions

This product is for research or for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

### Storage and Stability

Lyophilized LONG®R³IGF-I is known to be stable for three years when properly stored at 2 to 8 C.

	Format	Shelf-life (2 to 8 C storage)
85580C	Lyophilized	3 years

After reconstituting a solution of lyophilized LONG®R³IGF-I as prepared below, the product should be stored re-capped in the original vial at 2 to 8 C. It is imperative that the vial is re-capped properly to form an airtight seal, as the volatile nature of the acetic acid solution can result in evaporation and consequentially a concentration of the LONG®R³IGF-I in solution.

### Preparation Instructions

#### Lyophilized LONG®R³IGF-I Resuspension

1. Lyophilized LONG®R³IGF-I is supplied in an atmosphere of nitrogen at a slight vacuum (-25 kPa). Remove the metal cap from the glass vial and introduce an air filled syringe through the septum to equalize the pressure.
2. Add sufficient 100 mM acetic acid solution to the vial to achieve a concentration of 1 mg/mL LONG®R³IGF-I. Concentrations of 1 mg/mL or more are recommended.
3. Mix the solution thoroughly to ensure the peptide is completely dissolved. Proceed to filtration.

#### LONG®R³IGF-I Filtration

- Resuspend LONG®R³IGF-I, the liquid LONG®R³IGF-I or media containing LONG®R³IGF-I at the working concentration may be filtered through a low protein-binding membrane such as Polyvinylidene Difluoride (PVDF) or Polyethersulfone (PES) with a pore size of 0.22 µm.

### Addition to Cell Culture Medium

The recommended final concentration range of LONG®R³IGF-I for use as a growth factor supplement in cell culture is 10 - 100 µg/L. Given that the optimum concentration varies depending upon the cell line, clone and the particular media formulation, it is recommended that a titration of LONG®R³IGF-I be performed for each cell line. In some instances a brief adaptation phase may be necessary for optimal cell culture performance. Inclusion of LONG®R³IGF-I in the culture feed is advantageous in some applications.

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## Adaptation

There are two basic methodologies for adapting cells to medium containing LONG<sup>®</sup>R<sup>3</sup>IGF-I.

### Direct Substitution

- Some clones do not require weaning and can be grown immediately in an alternative insulin-free medium that contains an appropriate quantity of LONG<sup>®</sup>R<sup>3</sup>IGF-I (10 - 100 µg/L, recommended starting at 50 µg/L).

### Gradual Weaning

- Gradually wean cells into medium containing LONG<sup>®</sup>R<sup>3</sup>IGF-I (recommended starting at 50 µg/L) by decreasing the insulin concentration in the medium at each passage. For example, if the starting concentration of insulin is 10 mg/L, reduce the concentration to 5 mg/L, then 2.5 mg/L, then 1.25 mg/L, etc., at each successive passage.
- During adaptation, you may notice a slight decrease in doubling times. Slower growth rates may not impact overall protein yield as LONG<sup>®</sup>R<sup>3</sup>IGF-I can increase culture viabilities and overall specific protein productivity.
- If the decrease in doubling time is significant — i.e. less than half the normal doubling time — repeat passaging in medium with the same LONG<sup>®</sup>R<sup>3</sup>IGF-I concentration until the cells recover.
- As the cells become adapted to lower concentrations of insulin (< 1.25 mg/L), periodically test the ability of the clone to grow in medium without insulin (i.e. medium only containing LONG<sup>®</sup>R<sup>3</sup>IGF-I). The point at which insulin can be fully removed from the medium will vary with each cell line.

## Characteristics

### Appearance

Lyophilized white/creamy powder

### Molecular weight

9108 - 9112 daltons

### Endotoxin

< 0.10 EU/µg protein

### Biological Activity

ED<sub>50</sub> < 10 ng/mL (stimulation of protein synthesis in L6 myoblasts)

### Identity/Consistency

Confirmed by N-terminal sequence analysis and HPLC (18 residues 95% single sequence)

### Purity

≥ 95% as determined by SDS-PAGE

## References

1. Francis, G., et al. *J. Mol. Endocrinol.* (1992) 8:213-223.
2. Thomas, J., Fung, V. *Animal Cell Technology: Products of Today, Prospects for Tomorrow* (1993) 91.
3. Morris, A., Schmid, J. *Biotechnol. Prog.* (2000) 16:693-697.

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