

Technical Bulletin

LONG®R³IGF-I Frequently Asked Questions

Is LONG®R³IGF-I made to cGMP standards?

Yes; LONG®R³IGF-I is manufactured according to cGMP standards. The production facility is regularly audited by European and US contract manufacturers and biopharmaceutical companies. SAFC Biosciences™ audits the facility every three years.

Are any animal-derived components used in the manufacture of LONG®R³IGF-I?

No; LONG®R³IGF-I is manufactured using a process that is free of animal-derived components. LONG®R³IGF-I is regulatory compliant and is currently used in the manufacture of several biopharmaceuticals approved by the FDA (United States), EMEA (Europe) and MHLW (Japan).

Can you describe the process by which LONG®R³IGF-I is prepared?

LONG®R³IGF-I is manufactured in a proprietary but conventional expression system. A frozen ampule of cells is taken from a validated working cell bank. The cells are fermented in a fed-batch system, in a fully defined media free of animal-derived components, to produce inclusion bodies which are harvested and dissolved. LONG®R³IGF-I is then refolded in redox buffer and purified by a four-stage chromatography step.

Four-stage downstream purification:

- Big beads Solid Phase (SP) cation exchange chromatography to capture LONG®R³IGF-I and remove host-cell impurities
- Reverse-phase chromatography (RPC 30) to remove host-cell contaminants and product-related impurities
- Fast flow SP cation exchange chromatography to further purify LONG®R³IGF-I
- Gel filtration (G25) using 100 mM acetic acid to remove urea and product-related impurities (aggregates)

The final step uses ultrafiltration to target the concentration range for dispensing and 5X diafiltration against 100 mM acetic acid to remove residual urea from the preceding gel filtration step. LONG®R³IGF-I is then dispensed and in the case of lyophilized Catalog No. 85580C, freeze dried.

What packaging sizes are available?

LONG®R³IGF-I is available from SAFC Biosciences as a freeze-dried powder in 1, 5, 10, 20 or 50 mg size vials, Catalog No. 85580C. A liquid formulation of LONG®R³IGF-I in 100 mM acetic acid is available in 5 mL and 100 mL sizes at a concentration of 1 mg/mL, Catalog No. 91590C.

How do I prepare LONG®R³IGF-I?

Liquid LONG®R³IGF-I is ready to use and there is no need to defrost or reconstitute. Simply open and dilute directly into cell culture media.

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.

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Important: Always reconstitute the entire vial *in situ* (in the vial) and then aliquot. Each vial contains the correct amount of peptide indicated on the label, but 15 - 20% more by mass, due to the residual moisture retained through the freeze-drying process. Do not attempt to estimate the peptide content by weight or gravimetric means as this will underestimate the actual peptide content.

How long is LONG[®]R³IGF-I stable?

Lyophilized LONG[®]R³IGF-I is known to be stable for three years when properly stored at 2 to 8 C. Liquid LONG[®]R³IGF-I in the original unopened vial is stable for 18 months when stored at 2 to 8 C. Formal ICH Q7A compliant studies are ongoing to further assess the long-term stability of liquid LONG[®]R³IGF-I.

	Format	Shelf-life (2 to 8 C storage)
85580C	Lyophilized	3 years
91590C	Liquid (1 mg/mL)	18 months (ongoing)

After reconstituting a solution of lyophilized LONG[®]R³IGF-I as prepared below, or after opening a vial of liquid LONG[®]R³IGF-I, 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.

Can I dissolve LONG[®]R³IGF-I in water or Phosphate Buffered Saline (PBS)?

No; the pH of the solution will not be optimal and may result in precipitation of the LONG[®]R³IGF-I.

Can I dissolve the powder directly into my cell culture media?

We do not recommend that the powder be dissolved in cell culture media.

Does LONG[®]R³IGF-I adhere to culture vessels and plumbing?

Like insulin and all small peptides, LONG[®]R³IGF-I can non-specifically adsorb to plastic, glass and stainless steel surfaces in low protein-containing media. Often this adsorption is minimal and does not affect overall cell culture performance. However, incorrect and inconsistent sample handling procedures can impact the accuracy of LONG[®]R³IGF-I detection.

To minimize non-specific adsorption of LONG[®]R³IGF-I:

- Reconstitute LONG[®]R³IGF-I stock solution at 1 mg/mL or greater.
- Always use filters with low protein-binding properties such as PVDF, PES or CA.

- Where applicable, add LONG[®]R³IGF-I as far down the media manufacturing process as possible, preferably to the fermentation tank directly.

To ensure consistency and accuracy in the measurement of LONG[®]R³IGF-I:

- Standardize the procedures for sampling and handling of cell culture media samples.
- Determine the optimal low protein-binding tube type for handling and storage of samples.
- Avoid sub-sampling and minimize repeated exposure to surfaces.
- The presence of a carrier protein in the media or tube can offer protection against non-specific adsorption.
- Ensure all samples have equilibrated to room temperature prior to analysis.

Filter Type	% loss of LONG [®] R ³ IGF-I
0.2 µm Cellulose Acetate (CA)	11%
0.2 µm Polyethersulfone (PES)	2%
0.1 µm Polyethersulfone (PES)	5%
0.2 µm Polyvinylidene Difluoride (PVDF)	< 1%
0.1 µm Polyvinylidene Difluoride (PVDF)	6%

Does LONG[®]R³IGF-I stick to filters?

Internal studies have shown that a small percentage of LONG[®]R³IGF-I is lost during filtration. The following table shows the amount of LONG[®]R³IGF-I lost on specific types of filter membranes.

Can I monitor the amount of LONG[®]R³IGF-I in my media, in-process or finished product samples?

Yes; an Enzyme-Linked Immunosorbent Assay (ELISA) is commercially available for determining LONG[®]R³IGF-I concentrations in samples. This kit is commercially available.

Will the addition of LONG[®]R³IGF-I to my media change the pH or the osmolality?

Because of the large dilution of sterile LONG[®]R³IGF-I into cell culture media, there should be no effect on pH or osmolality.

What cell types will respond to LONG®R³IGF-I?

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.

How much LONG®R³IGF-I should I add to my cell culture media?

The recommended concentration is 10 - 100 µg/L. It is not recommended to exceed 100 µg/L, and generally, lower concentrations work better than higher ones. A concentration of 50 µg/L is recommended as a starting point. A titration should be performed as the optimum concentration may vary based on the cell line or application.

How do I adapt my cells to medium containing LONG®R³IGF-I?

There are two basic methodologies for adapting cells to medium containing LONG®R³IGF-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®R³IGF-I (10 - 100 µg/L, recommended starting at 50 µg/L).

Gradual Weaning

- Gradually wean cells into medium containing LONG®R³IGF-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®R³IGF-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®R³IGF-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®R³IGF-I). The point at which insulin can be fully removed from the medium will vary with each cell line.

Should I use insulin as well as LONG®R³IGF-I in my media?

The effectiveness of LONG®R³IGF-I may be masked if used with commonly employed insulin concentrations (1 - 10 mg/L). However, the inclusion of lower concentrations of insulin (<1 mg/L) in conjunction with the recommended levels of LONG®R³IGF-I may result in beneficial synergistic effects in certain applications.

I tried LONG®R³IGF-I and it was no better than insulin. Why not?

Improvements in productivity are cell line and clone dependent. Factors affecting productivity include the cell type, the history of the cell line in terms of serum or insulin dependence and growing conditions such as media composition. Cell lines that are adapted to growth in serum-containing or serum-free medium containing insulin may require a longer adaptation to LONG®R³IGF-I before the full benefit can be achieved.

In general:

- Ensure that the correct concentration of LONG®R³IGF-I is tested (10 - 100 µg/L)
- LONG®R³IGF-I may not work well if used in the presence of high insulin concentrations
- Addition of physiological concentrations of insulin (2 - 5 µg/L) with LONG®R³IGF-I may have synergistic benefits

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Issued May 2008 T086
0106 0406 0207

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