LONG®R³ IGF-I Cell Culture Supplement FAQs

FAQ Sheet

LONG®R³ IGF-I manufacturing site and quality

LONG®R³ IGF-I is manufactured by Repligen Sweden AB. Repligen Sweden AB's quality management system is ISO 9001 certified and based on EU GMP. The production facility is regularly audited by European and US contract manufacturers and biopharmaceutical companies.

Are any animal-derived components used in the manufacture of LONG®R³ IGF-I?

No; LONG®R³ IGF-I is a recombinant protein produced in E. coli. It is specifically manufactured for mammalian cell culture using a process that is free of animal-derived components. LONG®R³ IGF-I 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®R³ IGF-I is prepared?

LONG®R³ IGF-I is manufactured in a proprietary, but conventional, expression system. Cells taken from a validated working cell bank are fermented in a fed-batch process using fully defined animal component-free media. Recombinant LONG®R³ IGF-I is then isolated from inclusion bodies, refolded in redox buffer, and purified by a four-stage chromatography process.

What are the available LONG®R³ IGF-I formats?

LONG®R³ IGF-I is available as a lyophilized powder, and a 1 mg/mL liquid formulation in 100 mM acetic acid.

What packaging sizes are available?

LONG®R³ IGF-I is available as a freeze-dried powder in 5 mg or 50 mg size vials. The 1mg/mL liquid formulation of LONG®R³ IGF-I is available in 5, 100, 250, 500, and 1000 mL sizes.

What is the release testing for LONG®R³ IGF-I?

Appearance, product identity (RP-HPLC, mass spectroscopy), purity, biological activity, bioburden and bacterial endotoxin analysis.

How do I prepare LONG®R³ IGF-I?

Lyophilized LONG®R³ IGF-I must be reconstituted prior to use.

1. The product is supplied in an atmosphere of nitrogen at a slight vacuum (-25 kPa).
2. Remove the metal cap from the glass vial and introduce an air-filled syringe through the septum to equalize the pressure.
3. Add sufficient 100 mM acetic acid solution to the vial to achieve a concentration of 1 mg/mL LONG®R³ IGF-I. We recommend keeping stock solution at ≥1 mg/mL.
4. Mix the solution thoroughly to ensure the peptide is completely dissolved.
5. Re-suspended LONG®R³ IGF-I, or media containing LONG®R³ IGF-I, 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.

Liquid LONG®R³ IGF-I is ready to use; there is no need to defrost or reconstitute. Simply open and dilute directly into cell culture media.

Does LONG®R³ IGF-I stick to filters?

Studies have shown that a small percentage of LONG®R³ IGF-I is lost during filtration. The following table shows the amount of LONG®R³ IGF-I lost on specific types of filter membranes.

Filter type	% loss of LONG®R³ IGF-I
0.2 µm Polyethersulfone (PES)	2%
0.1 µm Polyethersulfone (PES)	5%
0.2 µm Polyvinylidene (PVDF)	<1%
0.1 µm Polyvinylidene (PVDF)	6%
0.2 µm Cellulose Acetate (CA)	11%

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Can I dissolve LONG®R³ IGF-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®R³ IGF-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®R³ IGF-I adhere to culture vessels and plumbing?

Like insulin and all small peptides, LONG®R³ IGF-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 impmact the accuracy of LONG®R³ IGF-I detection.

To minimize non-specific adsorption of LONG®R³ IGF-I

- Reconstitute stock solutions of LONG®R³ IGF-I at 1 mg/mL or greater.
- Always use filters with low protein-binding properties such as PVDF or PES.
- Where applicable, add LONG®R³ IGF-I as far down the media manufacturing process as possible, preferable to the cell culture tank directly.

How long is LONG®R³ IGF-I stable?

Lyophilized LONG®R³ IGF-I is stable for five years when properly stored at 2 to 8° C. Liquid LONG®R³ IGF-I, in the original unopened vial, is stable for five years when stored at 2 to 8° C.

Reconstituted lyophilized LONG®R³ IGF-I, and open containers of liquid LONG®R³ IGF-I should be stored re-capped in the original vial at 2 to 8° C.

Once reconstituted, the solution can be aliquoted into smaller sizes (i.e., for single use) in and stored in LoBind eppendorf tubes at 2 to 8° C. LoBind tubes are recommended to prevent protein adsorption. Solutions stored under such conditions can be used for 12 months.

To ensure consistency and accuracy in the measurement of LONG®R³ IGF-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.

Can I monitor the amount of LONG®R³ IGF-I in my media, in-process or finished product samples?

Yes; an Enzyme-Linked Immunosorbent Assay (ELISA) is available from Repligen, Cat #9547-1, for determining LONG®R³ IGF-I concentrations in samples.

What is the effect of LONG®R³ IGF-I on cell culture?

LONG®R³ IGF-I has been shown to increase cell growth and overall volumetric productivity in CHO cell culture. In addition, LONG®R³ IGF-I may enhance protein production by reducing apoptosis and extending culture duration.

What cell types will respond to LONG®R³ IGF-I?

All cells that have a Type I IGF receptor, or are insulin sensitive, will potentially respond to LONG®R³ IGF-I. This group includes but is not limited to most commercially used cell lines such as Chinese Hamster Ovary cells (CHO), fibroblasts, hybridomas, embryonic stem cells (ES), natural killer cells (NK), mesenchymal stem cells (MSC), and hematopoietic stem cells (HSC).

Will the addition of LONG®R³ IGF-I to my media change the pH or the osmolality?

Because of the large dilution of sterile LONG®R³ IGF-I into cell culture media, there should be no effect on pH or osmolality.

How much LONG®R³ IGF-I should I add to my cell culture media?

The working concentration range for LONG®R³ IGF-I is 10 to 100 µg/L. We recommend starting with 50 µg/L then performing titrations to optimize concentrations with your cell line, process, and application.

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How to Adapt Cells

How do I adapt my cells to grow in media containing LONG®R³ IGF-I?

Direct or sequential adaptation are methodologies that can be used for weaning cells into LONG®R³ IGF-I containing media. Cells should be in mid-logarithmic growth phase and ≥90% viability before starting the adaptation process.

- **Direct**
  Some clones will not require weaning and can be grown immediately in media containing an appropriate quantity of LONG®R³ IGF-I (typically 10 to 100 µg/L).
- **Sequential**
  Less robust clones may need to be adapted to new media sequentially. Start by sub-culturing cells in a 25%:75% mixture of media with and without LONG®R³ IGF-I. When cell viabilities exceed 90%, and cell doubling times are stable, cells can be transitioned to the next media mixture (i.e., 50%:50%). If growth slows, or viability drops, cells should continue to be passaged in the same media until viability and doubling times stabilize.

Can I use LONG®R³ IGF-I if my media already contains insulin?

A combination of LONG®R³ IGF-I and insulin may be beneficial for some cell lines. We recommend the amount of LONG®R³ IGF-I be optimized for use with different cell lines, applications, and processes.

Step	% Starting Media	% Media with LONG®R³ IGF-I	Criteria to proceed to next step
1	75	25	Viability ≥90%, stable cell doubling time
2	50	50	
3	25	75	
4	0	100	

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