LONG® EGF Cell Culture Supplement FAQ

FAQ Sheet

What is LONG® EGF and what cell lines is it suitable for?

LONG EGF is a recombinant analogue of human epidermal growth factor (EGF) developed as a supplement for use in therapeutic cell culture applications as a like-for-like replacement for native EGF or recombinant human EGF (rhEGF). It comprises the human EGF amino acid sequence plus a 53 amino acid N-terminal extension peptide.
LONG EGF has applications in MSC, iPS, epithelial, fibroblast, HEK293, BHK-21, and MDCK cell lines. LONG EGF may also exhibit synergistic effects with LONG R^3 IGF-I in certain cell lines.

Is LONG EGF made to ISO 9001 standards?

Yes. LONG EGF is manufactured in accordance with the ISO 9001 quality management standard. The production facility undergoes regular audits by European and U.S. contract manufacturers as well as biopharmaceutical companies to ensure compliance and quality.

Are any animal-derived components used in the manufacture of LONG EGF?

No. LONG EGF is a cGMP grade recombinant protein produced in *E. coli*. It is specifically manufactured for mammalian cell culture using a process that is free of animal-derived components.

How much LONG EGF should I use in media?

The recommended concentration range is 10 – 50 µg/L. The maximum should not exceed 100 µg/L and, generally, lower concentrations work better than higher ones. As a starting point, we recommend developing a media with 50 µg/L.

Is it okay to dissolve the powder in media?

We do not recommend dissolving freeze-dried formulations in media.

At what concentration should we make the stock solution?

Concentrations of 1 mg/ml or more are recommended.

Is there product loss during membrane filtration?

As with all peptides, LONG EGF can stick to plumbing and filters in the absence of other protein components. We recommend that the supplemented media be sterile filtered with a low protein binding filter prior to use.

What is the recommended LONG EGF handling procedure?

1. Perform all activities in a controlled environment, such as a laminar flow cabinet using aseptic techniques.
2. When opening the vial, care should be taken to equilibrate the contents with ambient pressure.
3. An air-filled syringe may be introduced through the stopper to equalize the pressure before opening.
4. Reconstitute the vial to the recommended concentration of 1 mg/mL in 10 mM HCl.
5. Irrespective of the vial size, always reconstitute the entire vial *in situ*. Do not estimate the protein content by weight or gravimetric means as this will underestimate the actual protein content due to the residual moisture retained through the freeze-drying process.
6. Dilute further to 0.1 mg/mL using 10 mM HCl to achieve the working stock solution. Do not use phosphate buffered saline (PBS) or a buffer containing phosphate to dilute to the working stock, as this can cause the protein to precipitate out of solution.
7. Subsequent dilutions can be made in PBS or other buffered solutions including cell culture media.
8. If filtration is required, it is recommended to perform this using the 1 mg/mL or 0.1 mg/mL working stock solution through low-protein-binding filter membranes to avoid any non-specific binding of the protein during filtration.

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How do I adapt my cells to grow in media containing LONG EGF?

Direct or sequential adaptation are methodologies that can be used for weaning cells into LONG EGF 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 EGF (typically 10 to 100 µg/L).
- **Sequential (Table 1):** Less robust clones may need to be adapted to new media sequentially. Start by sub-culturing cells in a 75%:25% mixture of media with and without LONG EGF. When cell viability exceeds 90%, and cell doubling times are stable, cells can be transitioned to the next media mixture (i.e., 50%:50%) and then to the next mixture (i.e., 25%:75%) and, finally, 100% in new media. If growth slows, or viability drops, cells should continue to be passaged in the same media until viability and doubling times stabilize.

Table 1. Sequential Adaptation of Cells to LONG EGF-Containing Media

Step	% Starting Media	% Media with LONG EGF	Criteria to Proceed to Next Step
1	75	25	≥90% viability and stable cell doubling time
2	50	50	
3	25	75	
4	0	100	

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