LONG®R3 IGF-I Boosts IgG Production in CHO

Shashi Kudugunti, W. Roy Lin and James Rusche
Repligen Corporation, R&D, Waltham, MA 02453, USA

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

Summary

LONG®R3 IGF-I is a human IGF-1 analog containing a 13 amino acid N-terminal extension and substitution of an arginine for glutamic acid at position 3, which results in >1,000 fold reduced affinity for IGFBPs, thus enhancing its bioavailability and effectiveness in comparison to native IGF-I. LONG®R3IGF-I binds to and activates Type I IGF-1 receptor, leading to enhanced cell growth and productivity and it is also a common growth factor supplement used in CHO media as a replacement to insulin. LONG®R3 IGF-I is being used in several commercial antibody manufacturing processes .

An industrially relevant mammalian CHO DP12 cell line *(ATCC# CRL-12445™)* and cell culture conditions (media + feeds) were selected to evaluate and generate data on the efficacy of LONG®R3 IGF-I . The effect of LONG®R3 IGF-I on the productivity of antibody was compared between LONG®R3 IGF-I adapted and Non- LONG®R3 IGF-I adapted CHO DP12 cells. The fed-batch studies were carried out in shake flasks and results suggested that LONG®R3 IGF-I adapted cells produced two fold increase in the protein production than Non- LONG®R3 IGF-I adapted cells**.** In addition, the benefit to cost ratio was demonstrated to be high.

LONG®R3 IGF-I can be used to enhance productivity while maintaining a serum free chemically defined media formulation. This provides a robust and cost effective production process.

Method

Fed-Batch Method

A frozen vial from LONG®R3 IGF-I adapted CHO DP12 cells was thawed into shake flask containing CD OptiCHO medium with 100 ng/mL LONG®R3 IGF-I , 200nM Methotrexate and 4mM Glutamax. After 4-7 days (at cell density 5-8 e6cells/mL), the cells were inoculated into fed-batch shake flasks containing CD OptiCHO medium with 100 ng/mL LONG®R3 IGF-I , and 4mM Glutamax. A similar thawing method was used for the Non- LONG®R3 IGF-I adapted CHO DP12 cell line vial .

Feeding strategies were similar for both LONG®R3 IGF-I adapted and Non- LONG®R3 IGF-I adapted CHO DP12 cells as listed in the below table.

Fed-Batch Conditions	
Cell Lines	LONG®R3 IGF-I Adapted DP12 Cells Non-LONG®R3 IGF-I Adapted DP12 Cells
Media	CD OptiCHO™ Medium *(Thermo Fisher# 12681029)*
Media supplements	With and Without LONG®R3 IGF-I (100 ng/mL) Methotrexate (200 nM)* Glutamax (4 mM)
Feeds *Feed A (Thermo Fisher# A10234-01)* *Feed B (Thermo Fisher# A10240-01)*	Day3: Feed A (5%,) + Feed B (5%)
	Day6: Feed A (5%) + Feed B (5%)
	Day9: Feed A (5%) + Feed B (5%)
	Day12: Feed A (5%) + Feed B (5%)
Glucose Feeds	No additional glucose feeds (Feeds already contain high concentrations)
Seeding Density	0.4 E6 Cells/mL
Antibody	Human Anti-IL-8

Note*: Methotrexate was used only for seed expansion and not in the fed-batch shake flasks

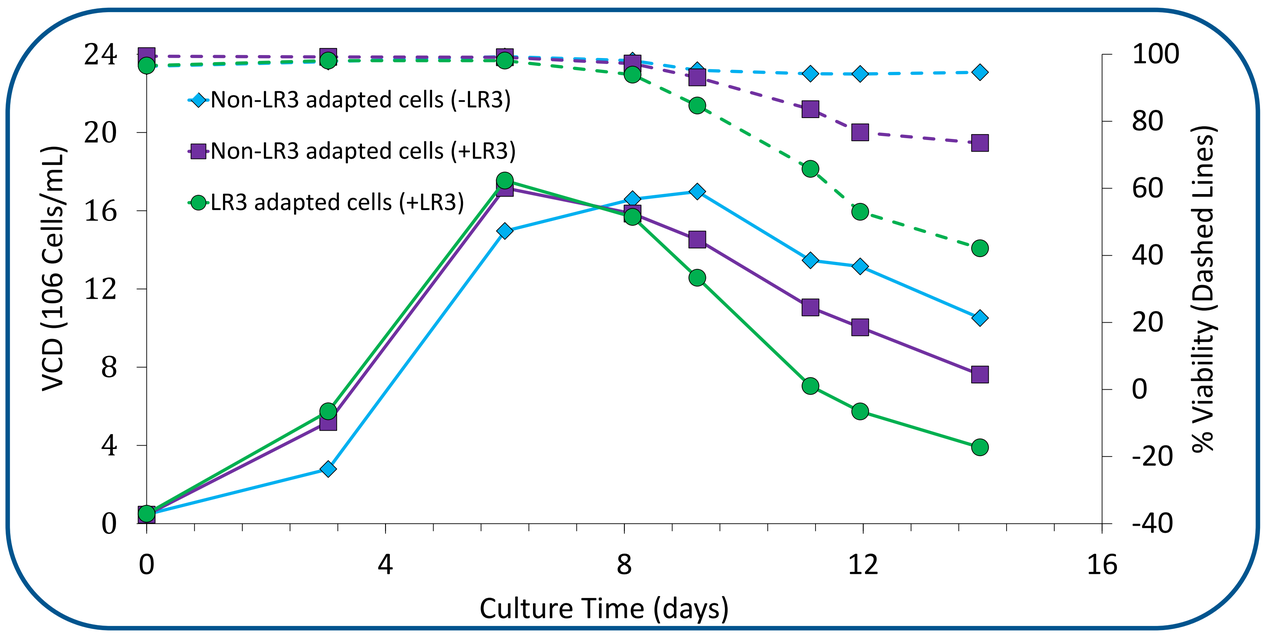
Fed-Batch Conditions

Flask No.	Fed-Batch Shake Flasks Description	LONG®R3 IGF-I
1	Non-LONG®R3 IGF-I adapted cells (-LONG®R3 IGF-I)	No
2	Non-LONG®R3 IGF-I adapted cells (+LONG®R3 IGF-I)*	Yes
3	LONG®R3 IGF-I adapted cells (+LONG®R3 IGF-I)	Yes

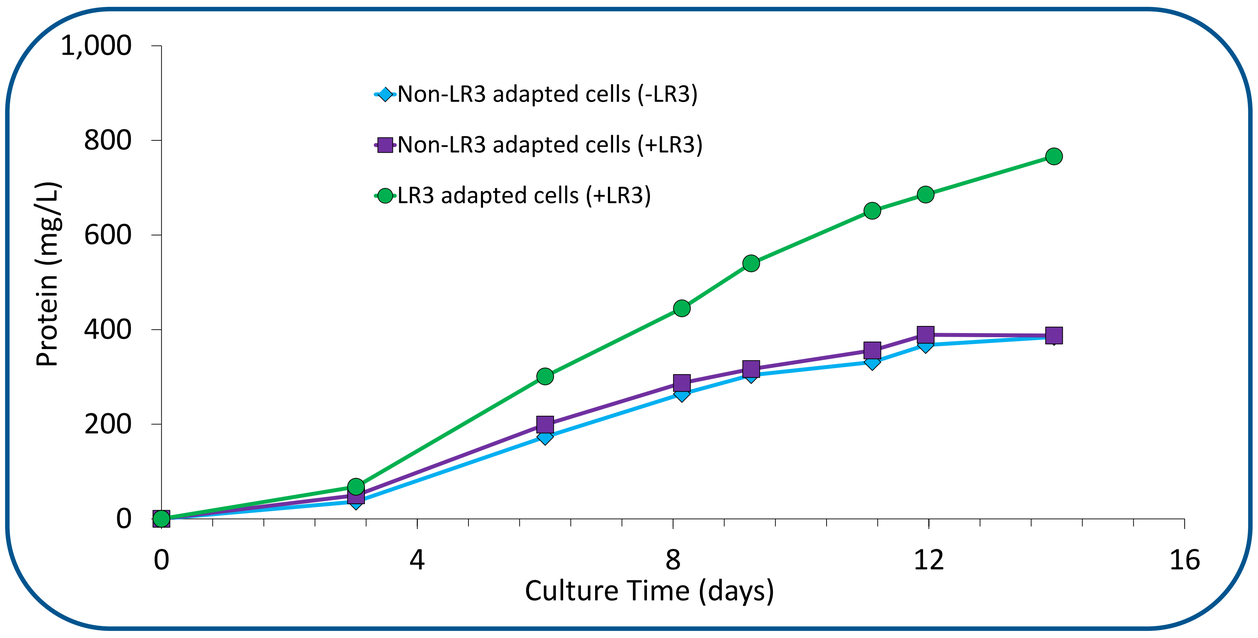
Note*: In shake flask 2, LONG®R3 IGF-I was added to fed-batch culture on Day 0 in spite of the cells being Non-LONG®R3 IGF-I adapted

Results

Viable Cell Density (VCD) and Viability

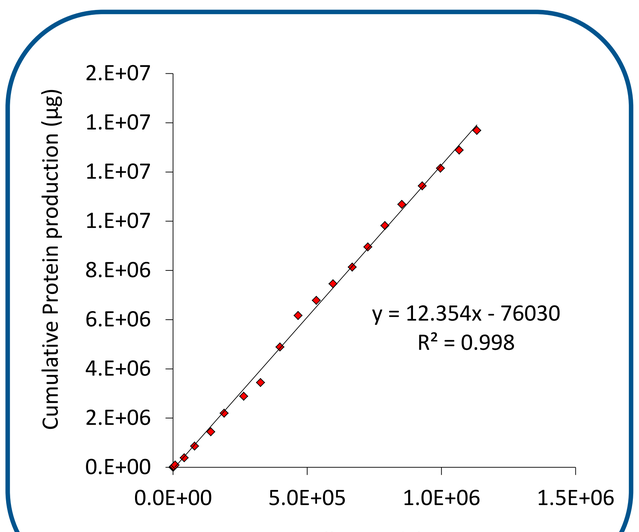


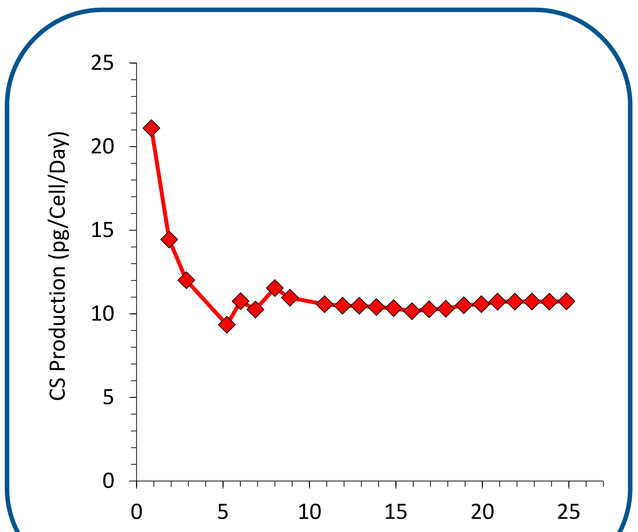
Protein Production



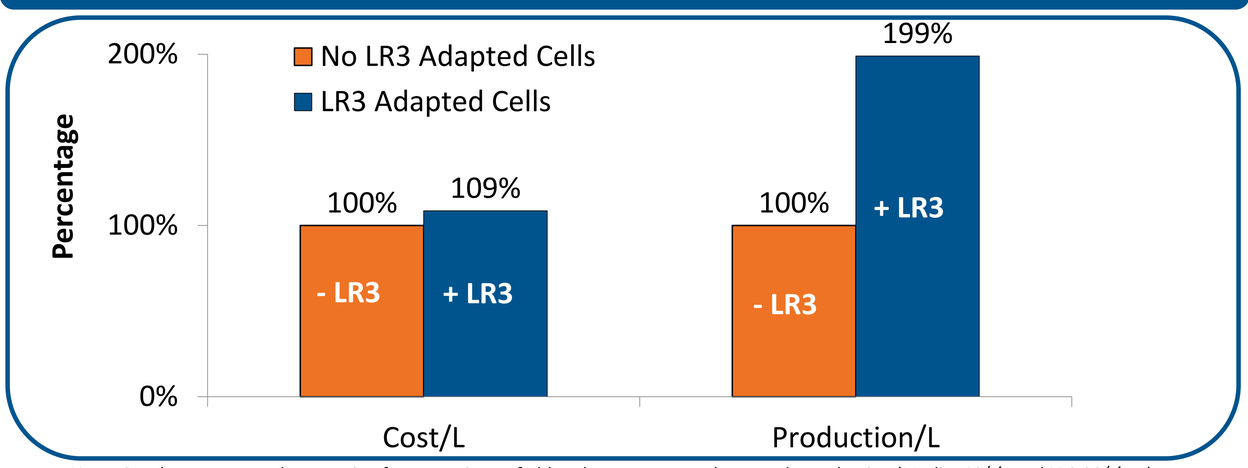
Cell Specific Production Rate of LONG®R3 IGF-I Adapted Cells

This perfusion experiment was conducted separately with LONG®R3 IGF-I adapted DP12 cells inoculated into a bioreactor connected to **Repligen ATF2 perfusion system** to determine cell specific production rate. The run was conducted without methotrexate and VCD was maintained at 40-60 E6 cells/mL.





Cost Benefit Analysis



Note: *Graph was prepared accounting for approximate fed-batch expenses on a large scale production (Media: 10$/L and LR3 20$/mg)*

Conclusions

- LONG®R3 IGF-I adapted CHO DP12 cells produced 2-fold increase in the protein yield than Non- LONG®R3 IGF-I adapted cells.
- LONG®R3 IGF-I adapted CHO cells produced 100% increase in IgG production with less than 10% in costs, when compared to the Non- LONG®R3 IGF-I adapted cells.
- Increased production of IgG was observed only after adapting the cells to LONG®R3 IGF-I . Supplementing the LONG®R3 IGF-I to the media without cell line adaption might not be helpful.
- LONG®R3 IGF-I adapted CHO DP12 cells did not lose cell specific production rate in a 25 day ATF perfusion culture.