Development and Characterization of a LONG©R³ IGF-1 ELISA

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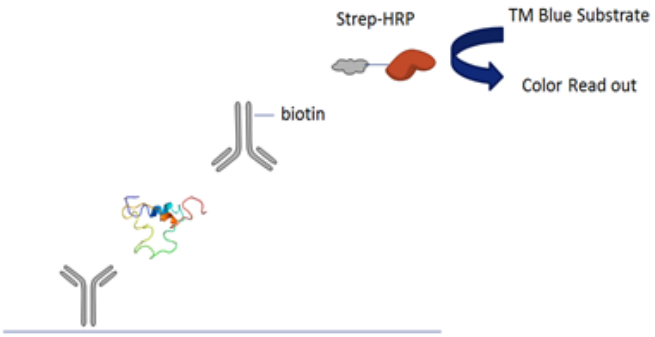
Abstract

LONG®R³ IGF-1 is a human IGF-1 analog containing a 13 amino acid N-terminal extension and a mutation at position 3. It activates the Type 1 IGF receptor, which is responsible for increasing cell growth and protein synthesis effects in CHO cells. A common growth factor supplement used in CHO media, insulin, also acts primarily through the IGF-R. Since LONG®R³ IGF-1 is manufactured under GMP and is used at low concentrations (10 – 100 ng/mL) in cell culture it represents a regulatory-friendly method of enhancing serum-free cell culture performance.

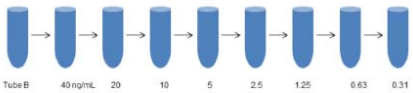
Quantitation of LONG®R³ IGF-1 is important when developing cell culture processes in order to optimize the concentration of LONG®R³ IGF-1 used and feeding strategies. Quantitation is also important when developing a purification process, in order to demonstrate clearance. An ELISA has been developed for the quantitation of LONG®R³ IGF-1 in media and drug substance samples. The assay uses a sandwich ELISA format with colorimetric detection. The performance characteristics of the assay are presented including accuracy, precision, linearity, LOQ, recovery from media, and range.

Schematic of LONG®R³IGF-1 ELISA



LONG®R³IGF-1 ELISA Instructions

Preparation of Standards (40 – 0.31 ng/mL)



Assay Instructions

Wash plate before use twice with 1x PBS

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Add 100 µL/well Standards, Samples, and QC Control (as applicable)
Incubate for 2 hours at 2-8°C
Wash three times with PBS-0.05% Tween 20

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Add 100 µL/well Detection Antibody to the plate
Incubate for 1 hour at 2-8°C
Wash three times with PBS-0.05% Tween 20

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Add 100 µL/well Streptavidin-HRP to the plate
Incubate for 30 minutes at 2-8°C
Wash two times with PBS-0.05% Tween 20
Wash once with 1x PBS

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Add 100 µL/well TM Blue to the plate
Develop then Stop with 100 µL/well 4 N Sulfuric Acid
Read plate at 450 nm

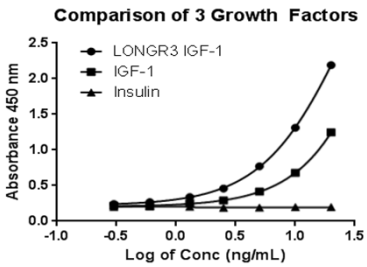
Accuracy, Precision, and Dilutional Linearity

Nominal Conc (ng/mL)	Composition	Assays	Recovery (Mean ± SD)
20	Diluent	8	21.1 ± 1.9
2.5	Diluent	8	2.5 ± 0.4
1.25	Diluent	8	1.2 ± 0.2
20	Conditioned media	1	20.4
2.5	Conditioned media	1	2.2
1.25	Conditioned media	1	0.9

For Dilutional Linearity, a sample was prepared at 80 ng/mL then 2-fold serially diluted down to 5 ng/mL. Dilutions of 40, 20, 10, and 5 ng/mL were run in the ELISA. Recoveries were within 70-130%.

Specificity

Specificity of the ELISA was measured by testing responses to the related molecules insulin and IGF-1. Results showed that the antibodies have no reactivity to insulin and only 35-40% reactivity with IGF-1.



Limits of Detection and Quantitation

The limit of quantitation is defined as the lowest concentration with recovery of 70-130% of nominal. The LOQ for the ELISA is 0.63 ng/mL.

The limit of detection was defined as the lowest concentration with a signal/noise ratio greater than 3. Signal/noise ratio was determined as the absorbance of the highest standard divided by the blank. The LOD for the ELISA is 0.31 ng/mL.

Conclusions

- Due to LONG®R³IGF-1's ability to increase cell growth and productivity, it is a useful supplement in manufacturing processes.
- In order to efficiently develop upstream and downstream processes it would be useful to quantitiate LONG®R³IGF-1 levels in culture and during purification.
- An ELISA kit has been developed that specifically, accurately, and precisely quantitates LONG®R³IGF-1.
- LONG®R³IGF1is produced in a validated, cGMP (ICH Q7) compliant, manufacturing process with no animal derived components; Final product is released by QC testing
- Ask us about applications for LONG®R³IGF-1 or visit growthfactors@repligen.com
  - CHO culture: cell line development, cell banking
  - Stem cell culture and regenerative medicine