

# Development and Characterization of LONG<sup>®</sup>R<sup>3</sup> IGF-I ELISA

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## Summary

LONG<sup>®</sup>R<sup>3</sup> IGF-I is an analog of IGF-I and has been engineered to deliver improved bioavailability. It binds to and activates Type I IGF-I receptor, leading to enhanced cell growth and productivity. A common growth factor supplement used in CHO media, insulin, also acts primarily through the IGF-R. Since LONG<sup>®</sup>R<sup>3</sup> IGF-I 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<sup>®</sup>R<sup>3</sup> IGF-I is an important aspect – especially for developing cell culture processes which are needed to optimize the concentration of the LONG<sup>®</sup>R<sup>3</sup> IGF-I. Quantitation of this growth factor will also be useful for feeding strategies.

Repligen has developed an ELISA kit (Cat# 9547-1) for the quantitation of LONG<sup>®</sup>R<sup>3</sup> IGF-1, both in media and in 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, recovery from media, LOQ, LOD, and range.

## LONG<sup>®</sup>R<sup>3</sup> IGF-1 ELISA Instructions

Wash plate before use twice with 1x PBS

Add Standards, Samples, and QC Control (as applicable) to the plate  
Incubate for 2 hours at room temp (without shaking)  
Wash three times with PBS-0.05% Tween 20

Add Detection Antibody to the plate  
Incubate for 1 hour at room temp (without shaking)  
Wash three times with PBS-0.05% Tween 20

Add Streptavidin-HRP to the plate  
Incubate for 30 minutes at room temp (without shaking)  
Wash two times with PBS-0.05% Tween 20  
Wash once with 1x PBS

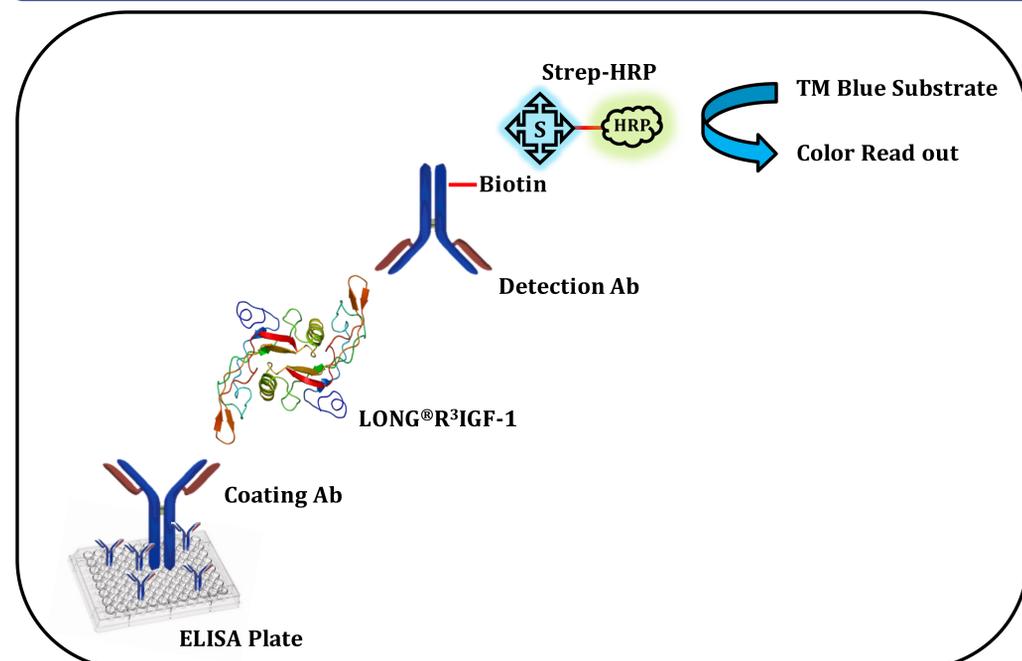
Add TM Blue to the plate  
Develop for 15 minutes at room temp (without shaking)  
Stop with 4 N Sulfuric Acid  
Read plate at 450 nm

## Accuracy, Precision and Dilutional Linearity

Nominal Conc (ng/mL)	Composition	# Assays	Recovery	
			Recovery (%)	RSD (%)
20	Diluent	12	91.0	9.3
5	Diluent	12	96.3	7.6
2.5	Diluent	12	105.1	8.4
20	Conditioned Media	1	90.3	NA
5	Conditioned Media	1	93.3	NA
2.5	Conditioned Media	1	96.6	NA

- For Dilutional Linearity, a sample was prepared at 40 ng/mL then 2-fold serially diluted down to 2.5 ng/mL. Dilutions of 20, 5, and 2.5 ng/mL were run in the ELISA. Recoveries were within 70-130%.

## Schematic of LONG<sup>®</sup>R<sup>3</sup> IGF-I ELISA



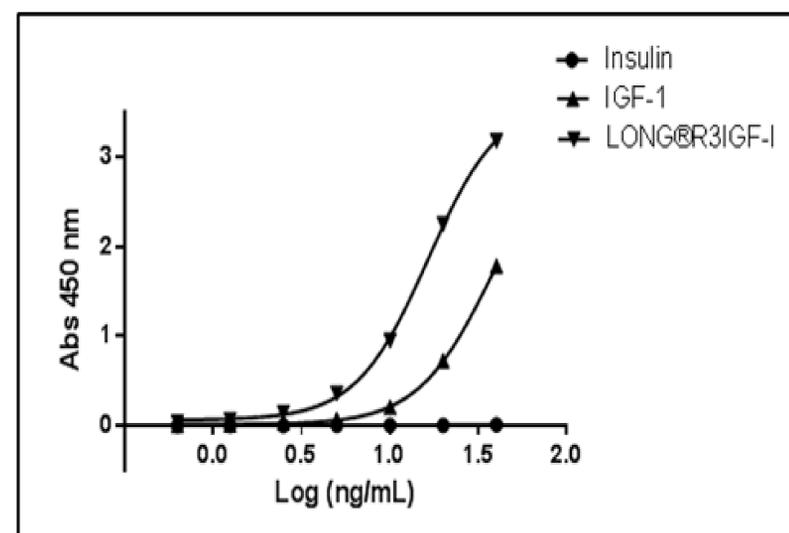
## Limit of Detection and Quantitation

- The limit of quantitation (LOQ) is defined as the LONG<sup>®</sup>R<sup>3</sup> IGF-I concentration corresponding to the mean of replicates of the zero standard plus 10 standard deviations. The LOQ for the ELISA is 1.25-2.5 ng/mL.
- The limit of detection (LOD) is defined as the LONG<sup>®</sup>R<sup>3</sup> IGF-I concentration corresponding to the mean of replicates of the zero standard plus 3 standard deviations. The LOD for the ELISA is 0.63-1.25 ng/mL.

## Specificity

- Specificity: The antibodies in ELISA kit have <5% reactivity to insulin at concentrations from 0.63 to 40 ng/mL and <35% reactivity to IGF-1 at concentrations from 0.63 to 20\* ng/mL.

**Note\*:** LONG<sup>®</sup>R<sup>3</sup> IGF-I 40ng/mL concentration was not considered in specificity as it was plateauing.



## Conclusions

- An ELISA kit has been developed that can be accurately, and precisely quantitates LONG<sup>®</sup>R<sup>3</sup> IGF-I in media samples.
- The ELISA kit has less than 5% cross reactivity to insulin at concentrations from 0.63 to 40 ng/mL and less than 35% cross reactivity to IGF-1 at concentrations from 0.63 to 20 ng/mL.