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HUMAN LONG®R³ IGF-1 ELISA

Catalog No: CKH194 **Quantity:** 1 Plate (1 x 96 tests) **Lot Number:** TBD **Expiration:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

Introduction:

LONG®R³ IGF-1 is an analog of Human IGF-1 which has been engineered for improved bioavailability compared to IGF-1. It binds to and activates the Type I IGF-1 receptor, leading to improved cell growth and productivity. 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.

This ELISA kit has been developed for the quantitation of LONG®R³ IGF-1 in media and drug substance samples. As shown in Figure 1, the plate is coated with a monoclonal antibody to LONG®R³ IGF-1 and then incubated with samples. A biotinylated detection antibody is added, followed by Streptavidin-HRP. The plate is incubated with TMB Substrate for color development and stopped with sulfuric acid. The signal is proportional to the amount of LONG®R³ IGF-1 in samples and standards.

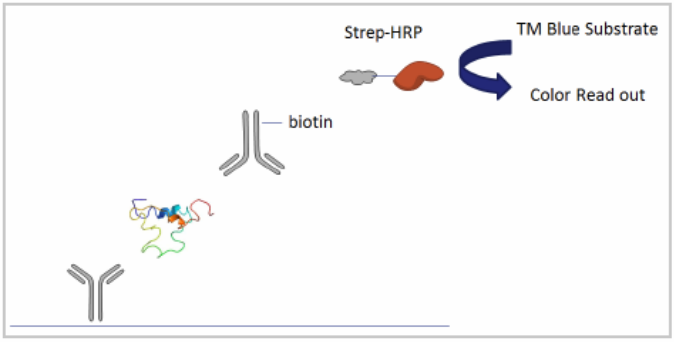

Figure 1. Schematic of LONG®R³ IGF-1 Sandwich ELISA

Reagents and Supplies Included:

Item	Description	Preparation	Storage
CKH194-P. 1 x 96-well pre-coated ELISA plate	Coated with anti-LONG®R³ IGF-1 antibody and dried	Use as per kit Instructions	2-8 °C
CKH194-T. LoBind Eppendorf Tubes (10)	Used for Standard Preparation	Ready to use.	Room Temperature
CKH194-A. Diluent	5x Concentrated solution	Dilute 5-fold in distilled water	2-8 °C
CKH194-B. LONG®R³ IGF-1 Standard	Lyophilized solid (1 mg)	Reconstitute in 1 ml 100 mM acetic acid then dilute as per Standard curve	2-8 °C
CKH194-C. Biotinylated Detection Antibody	Lyophilized solid	Reconstitute in 1 mL Washing Solution and then add to 10 mL Washing Solution (total volume 11 mL)	2-8 °C

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CKH194-D. Streptavidin-HRP	Liquid	Dilute 1000-fold in Washing solution	2-8 °C
CKH194-E. TMB Substrate	Liquid	Ready to use.	2-8 °C

***LONG**® is a trademark of Repligen Corporation.

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Reagents, Supplies, and Equipment Not Provided:

Reagent	Purpose	Amount Needed
Distilled Water	Dilution of Diluent (Reagent A)	16 mL
Glacial Acetic Acid	Reconstitution of LONG®R³ IGF-1 Standard	1 mL
PBS	Preparation of Washing Solution	2 L
Tween 20	Preparation of Washing Solution	0.5 mL
4 N Sulfuric Acid	Stop Solution	12 mL
Aluminum Foil	To protect plate from light	1 sheet
LoBind Eppendorf Tubes	Preparation of Sample (if sample needs to be diluted)	as needed
Reagent Reservoirs		4
Multichannel pipetter		1
Single channel pipetter		1 each for 2-20, 50-200, and 200-1000 µL volumes
Serological pipets		10 and 25 mL
15 mL tubes		
50 mL tubes		
Pipet tips		3 boxes
Plate Sealers		3
Vortexer		1
Microplate reader	For measurement of absorbance at 450 nm	1

Important Notes for Optimization of Kit Performance:

1. This ELISA kit is designed for quantitation LONG®R³ IGF-1. It has ~40% reactivity to IGF-1 and no reactivity to insulin.
2. LONG®R³ IGF-1 adsorbs to plastic and glass surfaces. Samples and standards should be prepared in low binding tubes such as LoBind Eppendorf tubes. The kit contains 10 LoBind tubes that may be used for preparation of standards.
3. In order to minimize adsorption, media samples should be collected into LoBind tubes.
4. When preparing samples, avoid multiple dilutions and minimize the number of transfers to prevent adsorption.
5. When withdrawing biotinylated LONG®R³ IGF-1 detection antibody (CKH194-C) or Streptavidin-HRP CKH194-D) from stock solutions, draw up the correct volume in the pipet tip, expel it entirely, and then draw up the correct volume again. This corrects for any adsorptive losses on tips.

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Plate Set-up:

Before starting the assay, the microtiter plate set-up should be defined. It is recommended to run standards and samples in triplicate. A representative plate setup is shown in Figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 40 ng/mL			Standard 0 (Blank)			Sample 8			Sample 16		
B	Standard 20 ng/mL			Sample 1			Sample 9			Sample 17		
C	Standard 10 ng/mL			Sample 2			Sample 10			Sample 18		
D	Standard 5 ng/mL			Sample 3			Sample 11			Sample 19		
E	Standard 2.5 ng/mL			Sample 4			Sample 12			Sample 20		
F	Standard 1.25 ng/mL			Sample 5			Sample 13			Sample 21		
G	Standard 0.63 ng/mL			Sample 6			Sample 14			Sample 22		
H	Standard 0.31 ng/mL			Sample 7			Sample 15			Sample 23		

Figure 2. LONG®R^3 IGF-1 Example Plate Set-up

Reagent Preparation:

100 mM acetic acid:

Add 100 mL of purified water to an appropriately sized bottle, followed by 575 µL of glacial acetic acid. Mix by shaking to generate 100 mM acetic acid solution. Solution may be scaled down as needed.

1x Diluent:

Add 16 mL of purified water to a conical tube followed by 4 mL of CKH194-A. Mix the tube by inverting 10 times to ensure thorough mixing. Vortexing is not recommended to avoid foaming.

PBS-Tween 20 Wash Solution:

1 L of Wash Solution should be prepared for testing a complete plate. Add 1 L of PBS to an appropriately sized bottle, followed by 500 µL Tween 20. Mix by swirling or inversion.

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LONG®R3 IGF-1 Reference Standard Preparation:

Note that LoBind tubes must be used for all dilutions of LR3 Reference Standard.

Label one Eppendorf® LoBind 1.5 mL tube as Tube B. The final concentration in Tube B will be 5 µg/mL. Label eight Eppendorf® LoBind 1.5 mL tubes with the concentrations of standard used in the ELISA: 40, 20, 10, 5, 2.5, 1.25, 0.63, and 0.31 ng/mL.

1. Reconstitute LONG®R^3 IGF-1 Reference Standard (CKH194-B), by adding 1 mL of 100 mM acetic acid to the lyophilized Reference Standard vial for a final concentration of 1 mg/mL. Mix by inverting 10 times. *Reconstituted reference standard can be stored for up to 1 month at 2-8 °C.*
2. Let sit for 15 minutes before use.
3. Prepare Tube B by adding 5 µL of reconstituted Reference Standard to 995 µL of 100 mM acetic acid for a final concentration of 5 µg/mL.
4. Mix tube by inversion after addition.
5. Prepare the 40 ng/mL standard by adding 8 µL of Tube B to 992 µL of 1x Diluent for a final concentration of 40 ng/mL. Mix tube by inverting ~10 times.
6. Use the 40 ng/mL tube as the starting point for 2-fold serial dilutions. Add 500 µL of 1x Diluent to each of the tubes labeled 20, 10, 5, 2.5, 1.25, 0.63 and 0.31 ng/mL. Transfer 500 µL from the 40 ng/mL tube to the 20 ng/mL tube. Mix tube by inverting ~10 times. In the same manner, transfer 500 µL from the 20 ng/ml tube to the 10 ng/mL tube and so on down to the 0.31 ng/mL tube (see Figure 3 below). *Store tubes at room temperature and use on the same day as preparation.*

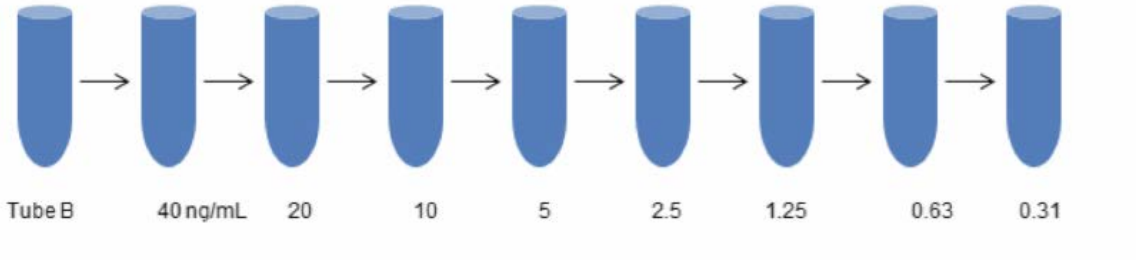

Figure 3: Preparation of Standard

Sample Preparation:

Samples that contain LONG®R^3 IGF-1 within the standard curve range (≤ 40 ng/mL) may be assayed without dilution.

Samples that contain greater than 40 ng/mL LONG®R^3 IGF-1 should be diluted in 1x Reagent A before analysis. Samples should be diluted in LoBind tubes using a minimum number of dilution steps.

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ELISA Procedure:

1. Remove the LONG®R^3 IGF-1 ELISA plate (CKH194-P) and TMB Substrate (CKH194-E) from 2-8 °C storage and allow it to equilibrate to room temperature. Ensure samples are prepared before proceeding.
2. Wash the LONG®R^3 IGF-1 ELISA plate twice with 1x PBS Buffer then dump or aspirate liquid from the wells and pound dry the inverted plate on clean paper towels.
3. Transfer of standards and samples to the plate should be done efficiently without pausing. Binding of the LONG®R^3 IGF-1 to the plate begins as soon as it is added to the well, so time to add standards and samples should be minimized.
4. Transfer 100 µL of the 40 ng/mL the LONG®R^3 IGF-1 Reference Standard (CKH194-B) to wells A1-A3. The same pipet tip should be used to transfer to all three wells. Discard the pipet tip and replace with a new one for the next transfer step. In the same manner transfer the remaining standards to the plate, including the blank. Transfer 100 µL of each Sample to the plate in the same manner as the standards.
5. After the Reference Standards and Samples have been added to the plate, cover the plate with a plate sealer.***** Incubate at room temperature for 2 hours.
6. Following the incubation, dump or aspirate the solution from the wells. Wash the wells three times with PBS - Tween 20 Wash Solution. Remove excess liquid or bubbles from each well by firmly tapping the plate dry on clean paper towels.
7. Prepare Detection Antibody Solution by adding 10 mL of the PBS-Tween 20 Wash Solution to a conical tube. Add 1 mL of PBS-Tween 20 Wash Solution to a vial of the LONG®R^3 IGF-1 ELISA Lyophilized Detection Antibody (CKH194-C). Mix by inverting ~10 times.
8. Transfer the reconstituted Detection Antibody quantitatively to the conical tube. Rinse the vial of Detection Antibody with 1 mL of solution from the 15 mL tube. Detection Antibody Solution must be used on the same day as it is prepared.
9. Using a 12-channel pipette, add 100 µL of Detection Antibody Solution to all wells on the plate. Cover the wells with a plate sealer.***** Incubate at room temperature for 1 hour.
10. After 1 hour incubation, dump or aspirate the conjugate solution from the wells, wash the wells three times with PBS-Tween 20 Wash Solution, and pound dry.
11. Prepare Streptavidin-HRP Solution by adding 12 mL of PBS-Tween 20 Wash Solution to a conical tube, followed by 12 µL of Streptavidin-HRP Conjugate (CKH194-D). Mix by inverting ~10 times.
12. Using a 12-channel pipette, add 100 µL of Streptavidin-HRP Solution to all wells on the plate. Cover the plate with a plate sealer and aluminum foil.***** Incubate at room temperature for 30 minutes.
13. After the 30 minute incubation, dump or aspirate the conjugate solution from the wells and pound dry. Wash the plate 2 times with PBS-Tween 20 Wash Solution and once with 1x PBS. Pound dry on clean paper towel.
14. Ensure that TMB-Peroxidase Substrate (CKH194-E) is at room temperature before use. Using a multi-channel pipette, add 100 µL of TMB-Peroxidase Substrate to each of the wells. Cover the plate with a sealer.*****
15. Incubate the plate for **15 minutes**. Immediately stop the reaction by adding 100 µL of 4N Sulfuric Acid to each well in the same order as for the TMB-Peroxidase Substrate. This will cause the solution to turn yellow.
16. Using a Plate Reader, read the plate at 450 nm. Read the plate immediately, no longer than within ten minutes of stopping the reaction.

* To ensure even dispersion, it is recommended to gently rotate the plate by hand about 10 times.

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Calculation of Results:

1. Fit the standard curve to a 4-parameter fit. A typical standard curve is shown in Figure 4.
2. Calculate the concentration of LONG®R^3 IGF-1 in each Sample using the Standard Curve. Be sure to account for any dilution factors.

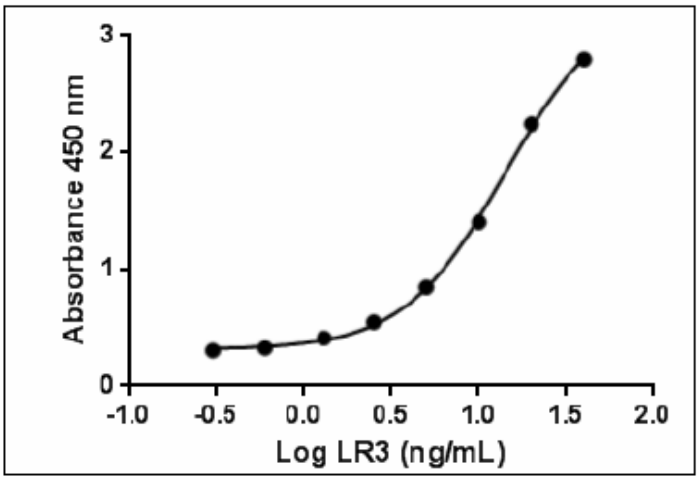

Figure 4. Representative LONG®R^3 IGF-1 Standard Curve.
(example only – do not use)

Assay Procedure Quick Reference:

Wash plate before use twice with 1x PBS.

↓

Add 100 µL/well Standards and Samples to the plate.*****
Incubate for 2 hours at room temperature.
Wash three times with PBS-0.05% Tween 20.

↓

Add 100 µL/well Detection Antibody to the plate.*****
Incubate for 1 hour at room temperature.
Wash three times with PBS-0.05% Tween 20.

↓

Add 100 µL/well Streptavidin-HRP to the plate.*****
Incubate for 30 minutes at room temperature.
Wash two times with PBS-0.05% Tween 20.
Wash once with 1x PBS.

↓

Add 100 µL/well TMB Substrate to the plate.*****
Develop for 15 minutes and then Stop with 100 µL/well 4 N Sulfuric Acid.
Read plate at 450 nm.

***** To ensure even dispersion, it is recommended to gently rotate the plate by hand about 10 times.

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Frequently Asked Questions:

1. **Can I use this ELISA to quantitate IGF-1 or Insulin as well as LONG®R³ IGF-1?**
   The antibodies used in the LONG®R³ IGF-1 ELISA do not react at all with insulin and only have ~40% reactivity with IGF-1. Therefore, it is not possible to quantitate insulin or IGF-1 using this assay.

2. **How can I store reconstituted Reference Standard?**
   Reconstituted reference standard can be stored for up to 1 month at 2-8 °C.

3. **How should I store Samples to minimize adsorptive losses?**
   LONG®R³ IGF-1 adsorbs to plastic and glass surfaces in the absence of other proteins or surfactants. Ideally, samples should be collected into low binding tubes such as LoBind™ eppendorf tubes.

Troubleshooting:

Problem	Possible Cause	Remedy
There is no signal on the entire plate.	Detection antibody or Streptavidin-HRP may have been omitted or reagents added in wrong order.	Repeat assay verifying addition of all reagents.
There is very low signal on the entire plate.	Detection antibody or Streptavidin-HRP may have been added at incorrect dilutions.	Repeat assay verifying dilutions of reagents.
	Plate reader may be set to the incorrect wavelength.	Repeat assay verifying 450 nm wavelength.
There is no signal from my samples.	Samples may not contain LONG®R³ IGF-1. ELISA will not accurately quantitate IGF-1 or insulin.	Check sample preparation.
	Samples may have been over-diluted.	Check dilution factors.
	Samples may have adsorbed to collection tube.	Collect samples in LoBind tubes.
There is high well-to-well variability.	Plate washing may have been inconsistent.	Repeat assay verifying number of washes and check for washing of each well or column.
	Pipetting may have been inaccurate.	Check accuracy of pipets.
There is high signal on the entire plate.	Plate washing may have been insufficient.	Repeat assay verifying number of washes and check for washing of each well or column.
	Detection antibody or Streptavidin-HRP may have been added at incorrect dilutions.	Repeat assay verifying dilutions of reagents.
	Re-use of plate sealers or reagent reservoirs may have resulted in HRP contamination.	Use new plate sealers and reservoirs.

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