TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Caution: For Laboratory Use. A research chemical for research purposes only.

Human Insulin-like Growth Factor 1 (IGF1) Kit

Product No.: AL236 C/F

Lot specific kit information can be found at www.perkinelmer.com/COA

Material Provided

Format: AL236C: 500 assay points AL236F: 5 000 assay points

The number of assay points is based on an assay volume of 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

Product Information

Kit content: The kit contains 5 components: AlphaLISA Acceptor beads coated with an Anti-Analyte Antibody, Streptavidin-coated Donor beads, Biotinylated Anti-Analyte Antibody, lyophilized analyte and 10X AlphaLISA Immunoassay Buffer.
Assay microplates (96-, 384- or 1536-well plates) must be purchased separately (see page 3 for more details).

Storage: Store kit in the dark at +4˚C. Store reconstituted analyte at -20°C.

Stability: This product is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions. Note: Once reconstituted, the human IGF1 analyte is stable for at least 75 days at -20°C (see page 2: Reagents and Materials).

Application: This kit is designed for the quantitative determination of human IGF1 in serum, buffered solution or cell culture medium using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 150 pg/mL (see page 8: Assay Performance Characteristics).

Dynamic range: 150 – 100 000 pg/mL (see page 8: Assay Performance Characteristics).

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Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on an EnVision® HTS instrument using the High sensitivity protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and depending on assay conditions with no impact on LDL measurement.

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Precautions

- Only the AlphaScreen® Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous. Some analytes are from human source.
- Some analytes are present in saliva. Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Reagents and Materials

The reagents provided in the AlphaLISA kit are listed in the table below:

Kit components	AL236C (500 assay points)	AL236F (5 000 assay points)
AlphaLISA Anti-IGF1 Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Antibody Anti-IGF1 stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
AlphaLISA human IGF1 (1 µg), lyophilized analyte *	1 tube, clear cap	1 tube, clear cap
AlphaLISA Immunoassay Buffer (10X) **	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute human IGF1 in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes, if possible, or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. It has been demonstrated that reconstituted human IGF1 is stable for at least 75 days at -20°C. One vial contains an amount of human IGF1 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL236S).

** Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100 and 0.5% Proclin-300. Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).
Note: 10X buffer might be slightly yellow. However, this does not affect the assay results.

Once diluted, 1X AlphaLISA Immunoassay Buffer contains 25 mM HEPES, pH 7.4, 0.1% Casein, 1 mg/mL Dextran-500, 0.5% Triton X-100 and 0.05% Proclin-300.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	PerkinElmer Inc.	6050195
EnVision®-Alpha Reader	PerkinElmer Inc.	-

Protocols have been optimized for 50 µL assays in white OptiPlate™-384 microplates. Other assay volumes can be used with similar protocols and identical final AlphaLISA reagent concentrations:

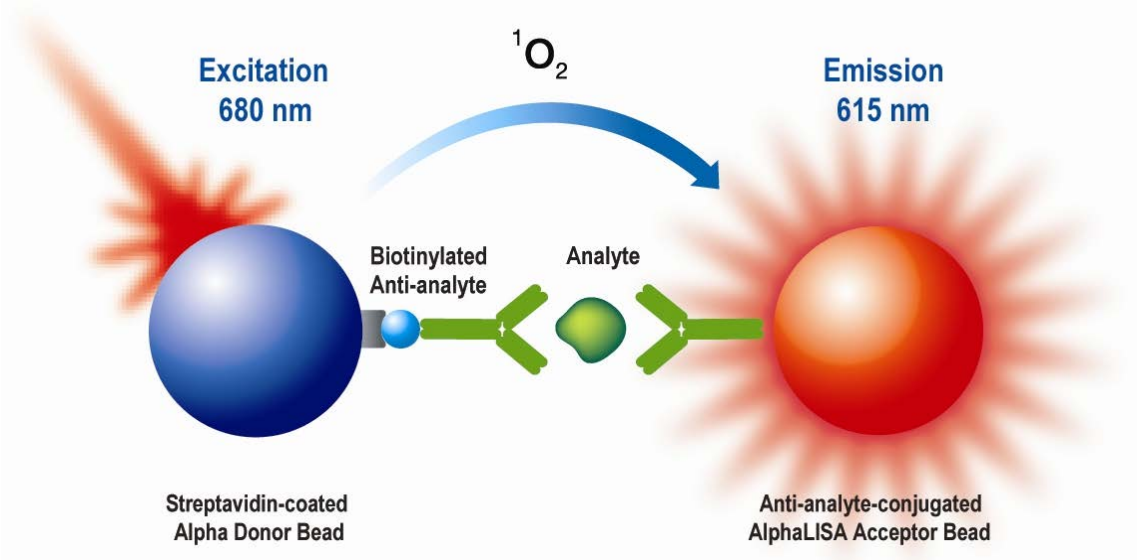
Format	# of data points	Total assay volume	Sample volume	AlphaLISA beads / Biotin Antibody MIX volume	SA-Donor beads volume	Plate recommendation
AL236C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	20 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL236F	5 000	50 µL	5 µL	20 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Analyte of Interest

Insulin-like Growth Factor 1 (IGF1 or Somatomedin C) is 70 amino acids in length after being fully processed. IGF1 is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion. IGF1, via binding to the IGF receptor, acts as natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication as well as a potent inhibitor of programmed cell death. IGF1 mediates many of the growth-promoting effects of growth hormone by promoting the incorporation of sulfate into cartilage in many normal tissues. The insulin-like growth factor pathway plays a major role in cancer cell proliferation, survival and resistance to anti-cancer therapies underlying the importance of IGF1 as a serum biomarker in various diseases such as diabetes and cancer.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated Donor beads while another Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (see figure below).



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Recommendations

General recommendations:

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to prewet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2 000 g, 10-15 sec). Resuspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added in the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the ALPHA option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.

Specific recommendations:

- AlphaLISA assays can be performed in cell culture medium with or without phenol red, with the following recommendations: if possible, avoid biotin-containing medium (e.g. RPMI medium) as lower counts and lower sensitivity are expected. It is not recommended to add FBS to the cell culture medium, since FBS strongly interferes with the assay. Casein (0.1%) was added to cell culture medium as carrier protein (e.g. MEM + 0.1% casein).
- When analyzing serum samples, perform the standard curve in 1X AlphaLISA Immunoassay Buffer and dilute the samples at least 10-fold before testing to fall within the assay dynamic range.

Protocol

High sensitivity protocol (2 incubation steps) – Dilution of standards in 1X AlphaLISA Immunoassay Buffer or cell culture medium supplemented with 0.1% casein

The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocol also includes testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.

The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

Use of four background points in triplicate (12 wells) is recommended when LDL (Lower Detection Limit) is calculated. One background point in triplicate (3 wells) can be used when LDL is not calculated.

IMPORTANT: PLEASE READ THE RECOMMENDATIONS ABOVE BEFORE USE

Steps for Preparing Reagents

The protocol described below is for one standard curve (48 wells) and samples (452 wells). Dilution of standards can be done in 1X AlphaLISA Immunoassay Buffer or cell culture medium supplemented with 0.1% casein. If interested in detecting both bound and unbound IGF1 from cell culture and serum samples, we recommend an acid dissociation step (see Acid Dissociation Protocol on page 8).

If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 2.5 mL of 10X AlphaLISA Immunoassay Buffer to 22.5 mL H_2O

2) Preparation of human IGF1 analyte standard dilutions:
Reconstitute lyophilized human IGF1 (1 µg) in 100 µL H_2O.
Prepare standard dilutions as follows (change tip between each standard dilution):

Tube	Vol. of human IGF1 (µL)	Vol. of diluent (µL) *	[human IGF1] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted human IGF1	90	1E-06	1 000 000
B	60 µL of tube A	140	3E-07	300 000
C	60 µL of tube B	120	1E-07	100 000
D	60 µL of tube C	140	3E-08	30 000
E	60 µL of tube D	120	1E-08	10 000
F	60 µL of tube E	140	3E-09	3 000
G	60 µL of tube F	120	1E-09	1 000
H	60 µL of tube G	140	3E-10	300
I	60 µL of tube H	120	1E-10	100
J	60 µL of tube I	140	3E-11	30
K	60 µL of tube J	120	1E-11	10
L	60 µL of tube K	140	3E-12	3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer, cell culture medium supplemented with 0.1% casein). At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 2.5X AlphaLISA Anti-IGF1 Acceptor beads + Biotinylated Antibody Anti-IGF1 MIX (25 µg/mL / 2.5 nM):
Add 50 µL of 5 mg/mL AlphaLISA Anti-IGF1 Acceptor beads and 50 µL of 500 nM Biotinylated Antibody Anti-IGF1 to 9 900 µL of 1X AlphaLISA Immunoassay Buffer. Prepare just before use.

4) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL): Keep the beads under subdued laboratory lighting.
Add 200 µL of 5 mg/mL SA-Donor beads to 12 300 µL of 1X AlphaLISA Immunoassay Buffer.

5) Samples: If applicable, dilute samples to be tested in diluent (e.g. 1X AlphaLISA Immunoassay Buffer or cell culture medium supplemented with 0.1% casein).

6) In a 96- or 384-well microplate:

Add 5 µL of each analyte standard dilution or 5 µL of sample

⇩

Add 20 µL of a 2.5X MIX (freshly prepared)
AlphaLISA Anti-Analyte Acceptor beads (10 µg/mL final) and Biotinylated Antibody Anti-Analyte (1 nM final)

⇩

Incubate 60 minutes at 23˚C

⇩

Add 25 µL of 2X SA-Donor beads (40 µg/mL final)

⇩

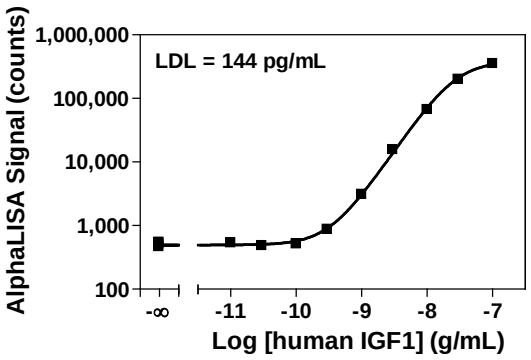
Incubate 30 minutes at 23˚C in the dark

⇩

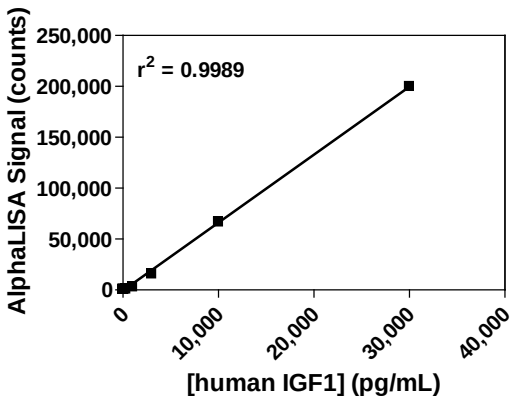
Read using EnVision-Alpha Reader

Typical results in 1X AlphaLISA Immunoassay Buffer

Log-Log scale:



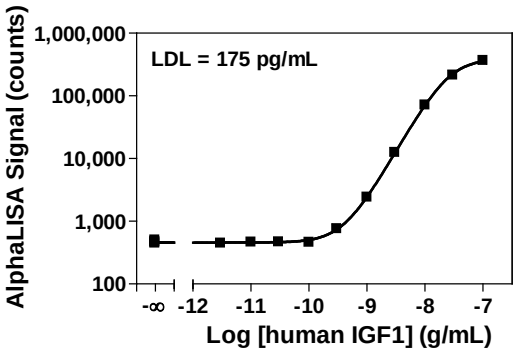
Linear-Linear scale (linear range):



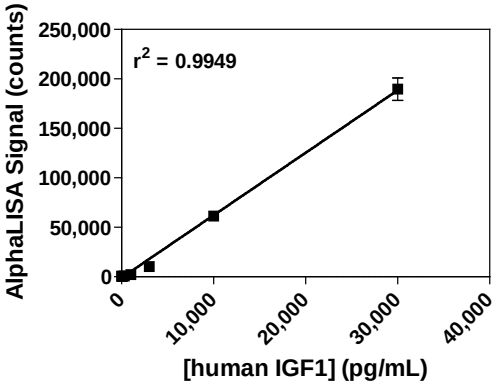
The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102.

Typical results in MEM + 0.1% casein

Log-Log scale:



Linear-Linear scale (linear range):



The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102.

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Interpreting the Data

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Acid Dissociation Protocol

The following protocol is recommended to dissociate all IGF1 from bound proteins in serum and cell culture samples. If using this protocol, a standard curve should also be run using the following buffer: 1:4:2 ratio of H_20, Acid disassociation buffer, and Neutralization buffer in place of the immunoassay buffer.

Acid dissociation buffer: 12.5% HCl, 2N, 87.5% EtOH
Neutralization buffer: 0.85M Tris, pH 11

For sample preparation:
Dilute samples 1:5 in Acid dissociation buffer (e.g. 200 µL sample + 800 µL Acid dissociation Buffer)
Incubate 30 minutes at 23°C
Spin @ 10,000 x g for 5 minutes
Take aliquot of supernatant and add Neutralization buffer- add 4 µL for every 10 µL of supernatant
e.g. 100 µL of supernatant + 40 µL of Neutralization buffer
Run 5 µL of neutralized sample/well

If neutralized samples contain IFG1 concentrations above the dynamic range of the kit, we recommend performing dilutions in the following buffer: 1:4:2 ratio of H_20, Acid dissociation buffer, and Neutralization buffer.

Assay Performance Characteristics

Sensitivity:

The LDL was calculated as described above. This value corresponds to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions.

- Average LDL is 150 pg/mL * (using 5 µL of analyte in AlphaLISA Immunoassay Buffer) (mean of 27 independent experiments).
- Average LDL is 188 pg/mL (using 5 µL of analyte in MEM + 0.1% casein) (mean of 8 independent experiments).

* Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 µL of analyte in a final assay volume of 50 µL).

Dynamic range: 150 – 100 000 pg/mL (in AlphaLISA Immunoassay Buffer)

Assay precision:

The following assay precision data were calculated from a total of 18 assays. Three operators performed three independent assays using two different kit lots. Each assay consisted of one standard curve and three control samples of high (A), medium (B) and low (C) concentration, assayed in triplicate. The assays were performed in 384-well format using AlphaLISA Immunoassay Buffer.

- Intra-assay precision:

The intra-assay precision was determined using a total of 18 independent determinations in triplicate for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 18)
A	33 561	1 707	5.1
B	3 382	122	3.6
C	1 083	31	2.8

- Inter-assay precision:

The inter-assay precision was determined using a total of 6 independent determinations with 9 measurements for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 6)
A	33 561	3 928	11.7
B	3 382	229	6.8
C	1 083	83	7.7

Human serum experiments:

In the following experiments, AlphaLISA Immunoassay Buffer was used as diluent in both the standard curve and dilution of samples. Additionally, all human serum samples tested were pre-diluted 10-fold with the diluent before being processed.

- Dilutional linearity:

The dilutional linearity was determined by serial dilutions of a pool of non-spiked human sera. The recovery was calculated using the 10-fold diluted sample as the 100% value. The average recovery from two independent measurements is reported.

Dilution Factor	% Recovery
1	100
2	103

- Serum sample values:

Frozen human serum samples were analyzed using the above stated conditions.

Number of samples	20
Number of samples with analyte concentration ≥ LDL	20
Average analyte concentration	107 ng/mL
Range of analyte concentration	54.0 - 142 ng/mL

Calibration:

Human Insulin-like Growth Factor-I (NIBSC/WHO First International Standard (code 02/254)) was tested using this kit: 1.0 µg of Standard corresponds to 0.93 µg of AlphaLISA IGF1.

Specificity:

Cross-reactivity of the AlphaLISA IGF1 Kit was tested using the following proteins at 0.3 µg/mL in AlphaLISA Immunoassay Buffer.

Protein	% Cross-reactivity
Mouse IGF1	0
Rat IGF1	0
Human IGF2	0
Human insulin	0

The possible interference from human Insulin-like Growth Factor-Binding Protein 3 (IGFBP3) was investigated. The human IGF1 was kept at a constant concentration (EC_{50} value of the standard curve). Human IGFBP3 was titrated into the assay. No interference was observed up to 30 ng/mL.

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