

TiterZyme® - EIA

> **human IGF-I**

Catalog # 900-150

96 Well Enzyme Immunoassay Kit

For use with human serum and plasma



All reagents should
be stored at 4°C.



Check our website
for additional
protocols, technical
notes and FAQs.



For proper perfor-
mance, use the in-
sert provided with
each individual kit
received.

Table of Contents

2 Introduction

2 Principle

3 Materials Supplied

4 Storage

4 Materials Needed but Not Supplied

5 Reagent Preparation

6 Sample Handling

6 Extraction Procedure

7 Assay Procedure

8 Calculation of Results

8 Calibration

9 Typical Results

10 Performance Characteristics

13 References

16 Limited Warranty

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Introduction

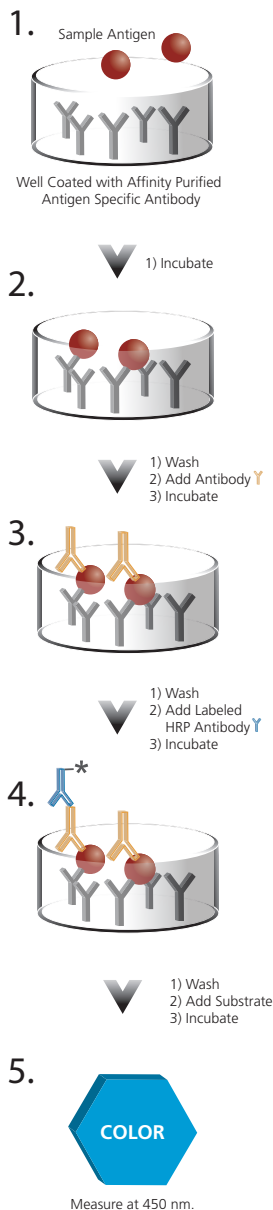
Assay Designs' human Insulin-like growth factor-I (IGF-I) TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of IGF-I in plasma and serum. Please read the complete kit insert before performing this assay.

Insulin-like growth factor-I (IGF-I) was originally known as Somatomedin C but was later changed to its current name due to its structural similarity to proinsulin^{1,2,3}. IGF-I has insulin-like effects⁴, but it appears to be involved in many other functions and has been linked to aging, nutrition, physical activity and cancer^{5,6,7}. While each of these is very interesting on its own, together they may shed light on the role that lifestyle plays in influencing cancer risk. High levels of circulating IGF-I and low levels of IGF binding protein-3 (IGFBP-3) are associated with increased risk of several cancers⁵. IGF-I can stimulate cell proliferation and inhibit apoptosis in many different kinds of cells^{5,8}.

IGF-I circulates in blood bound with IGFBPs. Six IGFBPs have been identified but the majority of circulating IGF-I is complexed with IGFBP-3. The IGF-I and IGFBP-3 exist in a complex with a third protein, named the acid-labile subunit. Serum and plasma samples must undergo an extraction procedure prior to immunoassay to separate the IGF-I from its binding proteins^{9,10}.

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody specific for IGF-I. The plate is then incubated.
2. The plate is washed, leaving only bound IGF-I on the plate. A yellow solution of polyclonal antibody to human IGF-I is then added. This binds the IGF-I captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the IGF-I polyclonal. The plate is again incubated.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. The substrate generates a blue color when catalyzed by the HRP.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of IGF-I in the sample



Materials Supplied

1. Assay Buffer 10 Concentrate
30 mL, Catalog No. 80-0648
Tris buffered saline containing detergents.
2. human IGF-I Standard
0.25 mL, Catalog No. 80-1448
One vial containing 60,000 pg/mL of recombinant human IGF-I.
3. 2N HCl
32mL, Catalog No. 80-1403
A 2N solution of hydrochloric acid in water.
4. Neutralizing Reagent 2
30mL, Catalog No. 80-1402
5. IGF-I Clear Microtiter Plate
One Plate of 96 Wells, Catalog No. 80-1394
A plate of break-apart strips coated with a mouse monoclonal antibody specific to IGF-I.
6. Wash Buffer Concentrate
105 mL, Catalog No. 80-1287
Tris buffered saline containing detergents.
7. human IGF-I EIA Antibody
11 mL, Catalog No. 80-1446
A yellow solution of rabbit polyclonal antibody to human IGF-I.
8. human IGF-I EIA Conjugate
11 mL, Catalog No. 80-1447
A blue solution of goat anti-rabbit IgG conjugated to horseradish peroxidase.
9. TMB Substrate
11 mL, Catalog No. 80-0350
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.
10. Stop Solution 2
11 mL, Catalog No. 80-0377
A 1N solution of hydrochloric acid in water.
11. IGF-I Assay Layout Sheet
1 each, Catalog No. 30-0229
12. Plate Sealer
3 each, Catalog No. 30-0012



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



The standard should be handled with care due to the known and unknown effects of the molecule.



2N HCl is caustic. Keep tightly capped.



Activity of conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.



Protect substrate from prolonged exposure to light.



Stop solution is caustic. Keep tightly capped.



All reagents should be stored at 4°C.

Storage

All components of this kit are stable at 4°C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Ethanol, ACS Grade, Sigma, Cat No. 02860.
3. Microcentrifuge.
4. Microcentrifuge tubes.
5. Precision pipets for volumes between 50 μ L and 1,000 μ L.
6. Repeater pipet for dispensing 100 μ L.
7. Disposable beakers for diluting buffer concentrates.
8. Graduated cylinders.
9. A microplate shaker.
10. Lint-free paper for blotting.
11. Microplate reader capable of reading at 450 nm.
12. Graph paper for plotting the standard curve.

Reagent Preparation

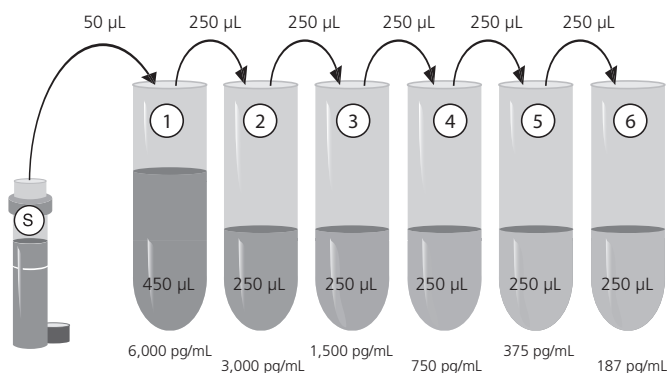
1. Wash Buffer

Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Assay Buffer 10

Prepare the assay buffer by diluting 50 mL of the supplied Assay Buffer 10 Concentrate with 450 mL of deionized water. This can be stored at 4°C until the kit's expiration or for 3 months, whichever is earlier.

3. human IGF-I Standards



Label six 12 x 75 mm polypropylene tubes #1 through #6. Pipet 450 µL of the assay buffer into tube #1. Pipet 250 µL of the assay buffer into tubes #2 through #6. Add 50 µL of the 60,000 pg/mL standard stock into tube #1 and vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

Diluted standards should be used within 30 minutes of preparation.

The concentration of IGF-I in tubes is labeled above.



Bring all reagents to room temperature for at least 30 minutes prior to opening.



Plastic tubes must be used for standard preparation.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.



Samples must be stored frozen at or below -20 °C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.



If the end user chooses to vary from the extraction procedure noted, it is up to the end user to determine the appropriate dilution of samples and assay validation.

Sample Handling

Most of the IGF-I in serum or plasma is complexed with binding proteins. Serum and plasma samples **must** be subjected to an extraction procedure to remove these binding proteins prior to assay. Following extraction, samples require an additional dilution prior to assaying.

Extraction Procedure

1. Combine 100% ethanol and the supplied 2N HCl at a ratio of 7:1.
2. In a microcentrifuge tube add the sample and ethanol:HCl mixture prepared in step 1 at a ratio of 1:5.
3. Vortex.
4. Incubate at room temperature for 30 minutes.
5. Microcentrifuge the sample for 5 minutes at 9,880 x g.
6. Transfer the supernatant to a new microcentrifuge tube.
7. Neutralize the supernatant with Neutralizing Reagent 2 at a ratio of 1:1.
8. Vortex.
9. If samples cannot be assayed immediately, store at -20°C.
10. Samples are now at a 1:12 dilution and require an additional 1:5.8 dilution with the assay buffer before running in the assay. This will bring samples to a final dilution of 1:70. **A minimum of a 1:70 final dilution is required to eliminate matrix interference in the assay.**

Example:

Mix 700 µL ethanol with 100 µL 2N HCl. Combine 50 µL of serum with 250 µL of ethanol:HCl mixture. Vortex, incubate, and centrifuge sample. 250 µL of Neutralizing Reagent 2 is added to 250 µL of the supernatant in a clean tube. The sample is now at a 1:12 dilution. 42 µL of the extracted and neutralized serum is combined with 203 µL of the assay buffer to yield a final dilution of 1:70 and a volume of 245 µL to run duplicate samples in the assay.

Sample Recoveries

IGF-I concentrations were measured in human serum and plasma samples. IGF-I was spiked into the extracted samples and diluted with the assay buffer, then assayed in the kit.

The following results were obtained:

Sample	Recommended Dilution	%Recovery
Extracted human plasma	≥1:70	102.8%
Extracted human serum	≥1:70	95.9%

Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used.

Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 μ L of the assay buffer into the S0 (0 pg/mL standard) wells.
2. Pipet 100 μ L of Standards #1 through #6 to the bottom of the appropriate wells.
3. Pipet 100 μ L of the samples to the bottom of the appropriate wells.
4. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
5. Empty the contents of the wells and wash by adding 400 μ L of wash buffer to every well. Repeat 4 more times for a total of 5 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 μ L of yellow antibody into each well except the blank.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm) at room temperature.
8. Wash as above (Step 5).
9. Add 100 μ L of blue conjugate to each well except the blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
11. Wash as above (Step 5).
12. Pipet 100 μ L of substrate solution into each well.
13. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
14. Pipet 100 μ L of stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.



Bring all reagents to room temperature for at least 30 minutes prior to opening.



All standards, controls, and samples should be run in duplicate.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



Pipet the reagents to the sides of the wells to avoid possible contamination.



Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.



Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Calculation of Results

Several options are available for the calculation of the concentration of IGF-I in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

2. Using linear graph paper, plot the average Net OD for each standard versus IGF-I concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Calibration

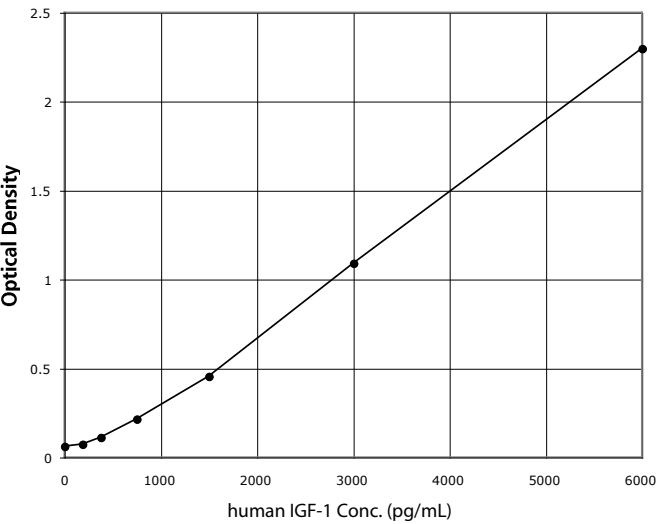
Calibration to the NIBSC/WHO IGF-I International Reference Reagent 87/518 has been determined. To convert sample values obtained in the TiterZyme® human IGF-I kit to this NIBSC/WHO IGF-I Standard, use the equation below.

$$\text{Obtained TiterZyme® human IGF-I value (pg/mL)} \times 1.25 = \text{NIBSC/WHO 87/518 value (pg/mL)}$$

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	IGF-I (pg/mL)
S0	0.062	0
S1	2.299	6,000
S2	1.091	3,000
S3	0.456	1,500
S4	0.217	750
S5	0.112	375
S6	0.075	187
Unknown 1	0.562	1,752
Unknown 2	0.154	554



Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at a concentration of 600,000 pg/mL. These samples were then measured in the assay.

Compound	Cross Reactivity
human IGF-I	100%
human IGF-II	0.11 %
IGFBP-2	<0.1 %
IGFBP-3	<0.1 %
IGFBP-4	<0.1 %
mouse IGF-I	<0.1 %
mouse IGF-II	<0.1 %
HGH	<0.1 %
HGF	<0.1 %
PDGF-BB	<0.1 %
human insulin	<0.1 %

Sample Values

The following human samples were tested for the presence of IGF-I.

Sample	# of Samples Tested	Range (ng/mL)	Mean (ng/mL)
serum	13	71 - 380	202
plasma-EDTA	12	136 - 264	200
plasma-Heparin	1	-----	143

Sensitivity

The sensitivity of the assay, defined as the concentration of IGF-I measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 48.5 pg/mL.

Linearity

A buffer sample containing IGF-I was serially diluted 1:2 in the assay buffer and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	---	4,554 pg/mL	---
1:2	2,250 pg/mL	2,254 pg/mL	100.2 %
1:4	1,125 pg/mL	1,135 pg/mL	100.9 %
1:8	562.5 pg/mL	637 pg/mL	113.2 %
1:16	281.25 pg/mL	315 pg/mL	112.0 %

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing IGF-I in a single assay.

pg/mL	%CV
220	8.9
514	4.9
1,739	3.6

Inter-assay precision was determined by measuring buffer controls of varying IGF-I concentrations in multiple assays over several days.

pg/mL	%CV
222	10.9
526	7.1
1,786	3.4

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MSDS (Material
Safety Data Sheet)
available online

Limited Warranty

Assay Designs, Inc. warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

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Kits for Charity™

Assay Designs has always been an active contributor to a number of local, national and international charities. We have broadened our charitable contributions by implementing a program called Kits for Charity™.

Each quarter, Assay Designs will feature a different non-religious and non-political charitable organization on our website. For each kit sold during this time period, we will make a monetary contribution to the featured charity. Please check our website for the current quarter's charity to see what organization your purchases are helping to support.

If you have any suggestions for future Kits for Charity™ recipients, please contact us at 800.833.8651 or 734.668.6113.

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