Product Manual

IGF-1 (human), ELISA kit

Catalog #: ADI-900-150

96 Well Enzyme-linked Immunosorbent Kit
For use with human serum and plasma

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

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

Product Manual

Please read entire booklet before proceeding with the assay.

TABLE OF CONTENTS

INTRODUCTION

The IGF-1 (human), ELISA kit is a complete kit for the quantitative determination of IGF-1 in plasma and serum. Please read the complete kit insert before performing this assay.

Insulin-like growth factor-1 (IGF-1) 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-1 has insulin-like effects[4], 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-1 and low levels of IGF binding protein-3 (IGFBP-3) are associated with increased risk of several cancers5. IGF-1 can stimulate cell proliferation and inhibit apoptosis in many different kinds of cells[5,8].

IGF-1 circulates in blood bound with IGFBPs. Six IGFBPs have been identified but the majority of circulating IGF-1 is complexed with IGFBP-3. The IGF-1 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-1 from its binding proteins[9,10].

PRINCIPLE

1. Samples and standards are added to wells coated with a monoclonal antibody specific for IGF-1. The plate is then incubated.
2. The plate is washed, leaving only bound IGF-1 on the plate. A yellow solution of polyclonal antibody to human IGF-1 is then added. This binds the IGF-1 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-1 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-1 in the sample.

SAFETY WARNINGS & PRECAUTIONS

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

Handle with care

- 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 know and unknown effects of the molecule.
- 2N HCl is caustic. Keep tightly capped.

Protect from light

- Activity of the 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.

MATERIALS SUPPLIED

1. **Assay Buffer 10 Concentrate**
   27 mL, Catalog No. 80-0648
   Tris buffered saline containing detergents.
2. **human IGF-1 Standard**
   0.25 mL, Catalog No. 80-1448
   One vial containing 60,000 pg/mL of recombinant human IGF-1.
3. **2N HCl**
   27 mL, Catalog No. 80-1403
   A 2N solution of hydrochloric acid.
4. **Neutralizing Reagent 2**
   30 mL, Catalog No. 80-1402
5. **IGF-1 Clear Microtiter Plate**
   One Plate of 96 Wells, Catalog No. 80-2438
   A plate of break-apart strips coated with a mouse monoclonal antibody specific to IGF-1.
6. **Wash Buffer Concentrate**
   100 mL, Catalog No. 80-1287
   Tris buffered saline containing detergents.
7. **IGF-1 (human) ELISA Antibody**
   10 mL, Catalog No. 80-1446
   A yellow solution of rabbit polyclonal antibody to human IGF-1.
8. **IGF-1 (human) ELISA Conjugate**
   10 mL, Catalog No. 80-1447
   A blue solution of goat anti-rabbit IgG conjugated to horseradish peroxidase.
9. **TMB Substrate**
   10 mL, Catalog No. 80-0350
   A solution of 3,3’,5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide.
10. **Stop Solution 2**
    10 mL, Catalog No. 80-0377
    A 1N solution of hydrochloric acid in water.
11. **IGF-1 Assay Layout Sheet**
    1 each, Catalog No. 30-0229
12. **Plate Sealer**
    3 each, Catalog No. 30-0012

STORAGE

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

OTHER MATERIALS NEEDED

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 µL and 1,000 µL.
6. Repeater pipet for dispensing 100 µL.
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

Bring reagents to room temp 30 minutes before 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.

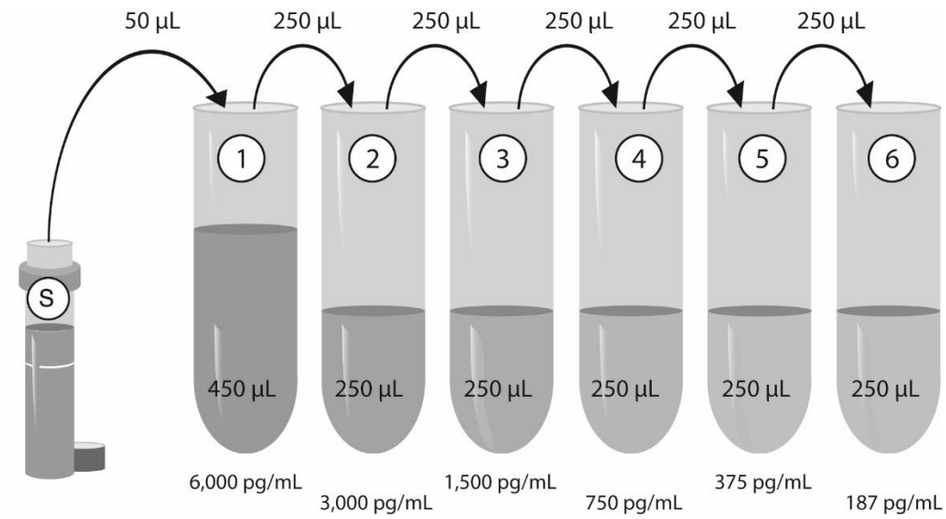
1. **Wash Buffer**

   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 30 mL of the supplied Assay Buffer 10 Concentrate with 270 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-1 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-1 in tubes is labeled above.

SAMPLE HANDLING

Most of the IGF-1 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-1 concentrations were measured in human serum and plasma samples. IGF-1 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

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate.

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 µL of the assay buffer into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #6 to the bottom of the appropriate wells.
3. Pipet 100 µL 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 µL 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 µL 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 µL 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 µL of substrate solution into each well.
13. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
14. Pipet 100 µL 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.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of IGF-1 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.

   Average Net OD = Average OD - Average Blank OD

2. Plot the average Net OD for each standard versus IGF-1 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-1 International Reference Reagent 87/518 has been determined. To convert sample values obtained in the IGF-1 (human) ELISA kit to this NIBSC/WHO IGF-1 Standard, use the equation below.

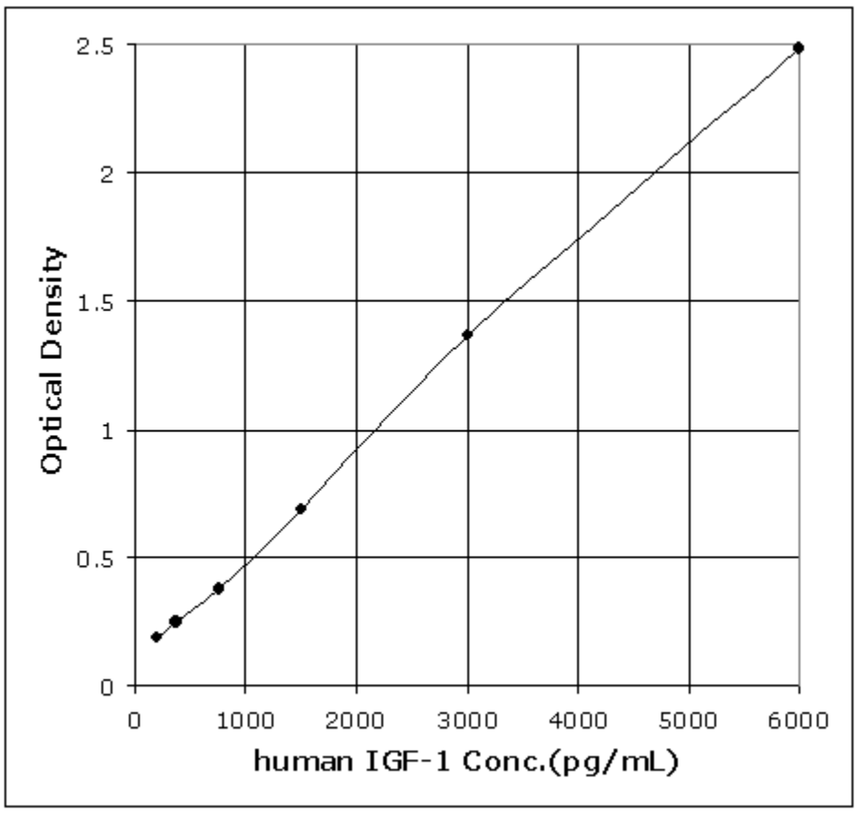
Obtained human IGF-1 value (pg/mL) x 1.25 =
NIBSC/WHO 87/518 value (pg/mL)

TYPICAL RESULTS

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

Sample	Net OD	IGF-1 (pg/mL)
S0	0.157	0
S1	2.488	6,000
S2	1.369	3,000
S3	0.684	1,500
S4	0.376	750
S5	0.249	375
S6	0.187	187
Unknown 1	0.721	1,570
Unknown 2	0.267	454

TYPICAL STANDARD CURVES



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-1	100%
human IGF-1I	0.11%
IGFBP-2	<0.1%
IGFBP-3	<0.1%
IGFBP-4	<0.1%
mouse IGF-1	<0.1%
mouse IGF-1I	<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-1.

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-1 measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 34.2 pg/mL.

Linearity

A buffer sample containing IGF-1 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	---
1:2	2,250	2,254	100.2
1:4	1,125	1,135	100.9
1:8	562.5	637	113.2
1:16	281.25	315	112.0

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing IGF-1 in a single assay.

Inter-assay precision was determined by measuring buffer controls of varying IGF-1 concentrations in multiple assays over several days.

Intra-assay		Inter-assay	
pg/mL	%CV	pg/mL	%CV
220	8.9	222	10.9
514	4.9	526	7.1
1,739	3.6	1,786	3.4

REFERENCES

1. E. Rinderknecht and R.E. Humbel. J. Biol. Chem. (1978) 253: 2769-2776.
2. M.E. Svoboda, et al. Biochemistry. (1980) 19:790-797.
3. B.T. Rudd. Ann. Clin. Biochem. (1991) 28:542-555.
4. E. Rinderknecht and R.E. Humbel. Proc. Natl. Acad. Sci. (1976) 73:2365-2369.
5. H. Yu =and T. Rohan. J. Natl. Cancer Inst. (2000) 92:1472-1489.
6. Y. H. Ibrahim and D.Yee. Growth Horm. & IGF Res. (2004) 14:261-269.
7. M.S. Lewitt. Diabetes Res. Clin. Pract. (1994) 23:3-15.
8. J.L. Jones and D.R. Clemmons. Endocr. Rev. (1995) 16:3-34.
9. R.E. Chestnut and V. Quarmby. J. Immun. Methods. (2002) 259:11-24.
10. J. Frystyk. Growth Horm. & IGF Res. (2004) 14:337-375.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com