Human Free IGF-I Immunoassay

Catalog Number DFG100

For the quantitative determination of human Free Insulin-like Growth Factor I (Free IGF-I) concentrations in EDTA plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

IGF-I (previously called somatomedin C) is a 7.6 kDa, 70 amino acid (aa) polypeptide with three internal disulfide bonds. The sequence of human IGF-I is identical to that of bovine and porcine IGF-I, and it is 70% identical to human IGF-II. IGF-I is a single-chain molecule with about 50% identity to the sequences of the A- and B-chains of human insulin. For reviews of IGF-I see references 1-3.

While IGF-I has very complex functions, it appears largely to mediate the actions of growth hormone. Thus, IGF-I is important in prenatal development, growth to adulthood and metabolic control. It induces amino acid uptake, protein synthesis and glucose utilization. It is an important mitogen and regulator of the cell cycle and apoptosis. IGF-I is produced primarily by hepatocytes, serving an endocrine function. It is also produced by many other cells, where it may act in an autocrine or paracrine manner. Serum levels of IGF-I have been reported to increase from birth to puberty, followed by a slow decline through adulthood (4).

There are two receptors for the IGFs; type I IGF receptor, which signals through a tyrosine kinase, and type II IGF receptor, which is identical to the mannose-6-phosphate receptor and may not signal. The action of IGF on its receptors is very complex, with control by at least six IGF-binding proteins (IGFBP-1 through IGFBP-6). IGFBP-3 binds over 90% of the total IGF in serum in a complex of IGF, IGFBP and an acid-labile subunit. This ternary complex greatly stabilizes IGF in the circulation, changing the half life from minutes to hours. In addition, IGFBPs modulate the action of IGF on the membrane receptors. Adding to the complexity is a family of proteases that act on IGFBPs, modifying their affinity for IGF or completely eliminating the IGFBPs. The interactions of IGF, IGFBP, IGFBP proteases, and IGF receptors are referred to as the IGF axis. The IGF axis affects many primary physiological and pathological processes, including development, growth, metabolic regulation, tumorigenesis, atherosclerosis and angiogenesis.

The Free IGF-I enzyme linked immunosorbent assay (ELISA) is a 1.75 hour solid-phase ELISA designed to measure human Free IGF-I in EDTA Plasma. It contains *E. coli*-expressed recombinant human IGF-I and has been shown to accurately quantitate the recombinant factor.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An avidin-coated microplate is simultaneously incubated with a biotinylated monoclonal antibody specific for Free IGF-I and standards, controls, or samples. The biotinylated antibody binds to the avidin-coated microplate, and any Free IGF-I binds to the biotinylated antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Free IGF-I is added to the wells. Following a wash to remove any unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of Free IGF-I bound. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
- To measure circulating free IGF-I, EDTA plasma samples should not be diluted prior to assay.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Avidin Microplate	894522	96 well polystyrene microplate (12 strips of 8 wells) coated with avidin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
IGF-I Standard	890775	Recombinant human IGF-I in a buffer with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	May be stored for up to 1 month at 2-8 °C.*
Free IGF-I Biotinylated Antibody	894521	3 mL of biotinylated monoclonal antibody against Free IGF-I with preservatives.	
Free IGF-I Conjugate Concentrate	894523	0.3 mL of concentrated monoclonal antibody against Free IGF-I conjugated to horseradish peroxidase with preservatives.	
Free IGF-I Conjugate Diluent	896000	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-66	895999	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	2 vials (6 mL/vial) of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human Free IGF-I Controls (optional; R&D Systems, Catalog # QC22).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Do not dilute prior to assay.*
Heparin and citrate plasma have not been validated for use in this assay.
Hemolyzed and lipemic samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

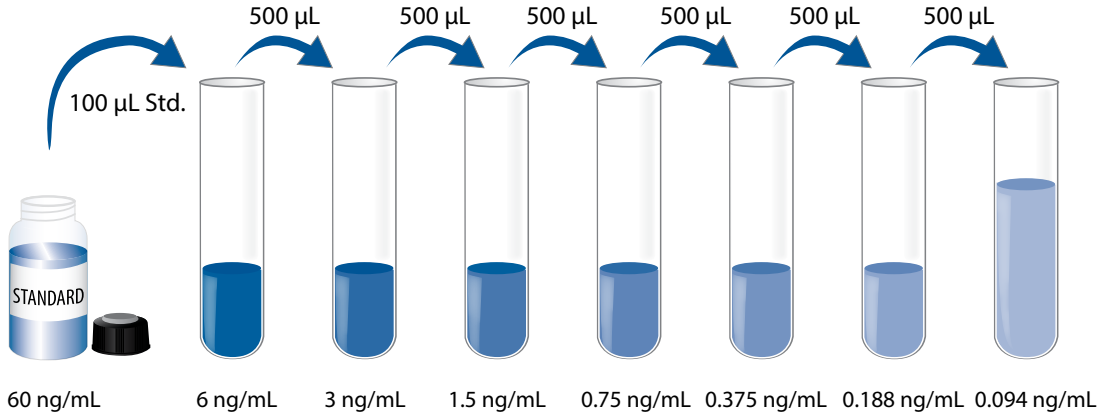
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Free IGF-I Conjugate Solution - Add 220 µL of Free IGF-I Conjugate Concentrate to 11 mL of Conjugate Diluent. This is a 50-fold dilution.

Note: *The Free IGF-I Conjugate Solution should be freshly diluted 10-15 minutes prior to use. Discard any unused Free IGF-I Conjugate Solution.* ***If a full plate is not being assayed, adjust volumes accordingly.***

IGF-I Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the IGF-I Standard with deionized or distilled water. This reconstitution produces a stock solution of 60 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6-66 into the 6 ng/mL tube. Pipette 500 µL of Calibrator Diluent RD6-66 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 6 ng/mL standard serves as the high standard. Calibrator Diluent RD6-66 serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 20 µL of the Free IGF-I Biotinylated Antibody to each well.
4. Add 50 µL of the standards, controls, and samples to the appropriate wells. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of the diluted Free IGF-I Conjugate Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 10 minutes at room temperature on the shaker. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

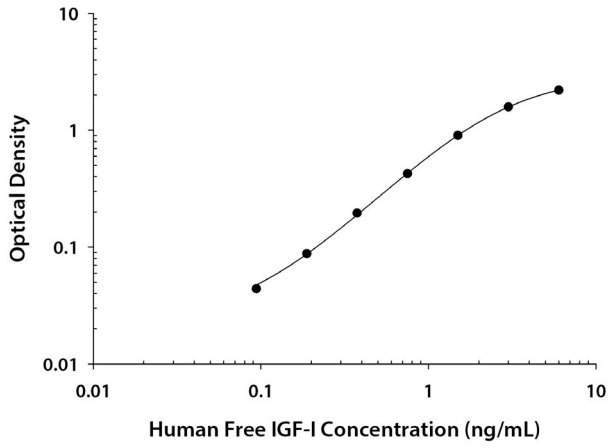
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Free IGF-I concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.025 0.025	0.025	—
0.094	0.068 0.069	0.069	0.044
0.188	0.111 0.115	0.113	0.088
0.375	0.217 0.222	0.220	0.195
0.750	0.443 0.457	0.450	0.425
1.5	0.908 0.948	0.928	0.903
3.0	1.583 1.629	1.606	1.581
6.0	2.205 2.243	2.224	2.199

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested eight times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in eight separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	8	8	8	8	8	8
Mean (ng/mL)	0.20	0.83	1.87	0.18	0.80	2.09
Standard deviation	0.01	0.03	0.07	0.02	0.08	0.13
CV (%)	5.0	3.6	3.7	11.1	10.0	6.2

RECOVERY

The recovery of samples spiked with IGF-I was evaluated.

Sample Type	Average % Recovery	Range
EDTA plasma (n=3)	107	93-118%

SENSITIVITY

The minimum detectable dose (MDD) of Free IGF-I is typically less than 0.015 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IGF-I produced at R&D Systems.

SAMPLE VALUES

EDTA Plasma - Samples from apparently healthy volunteers were evaluated for the presence of Free IGF-I in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
EDTA plasma (n=42)	0.503	93	ND-0.990

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human Free IGF-I.

The factors listed below were prepared at 200 ng/mL and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:

IGF-II
IGFBP-1
IGFBP-2

Other factors:

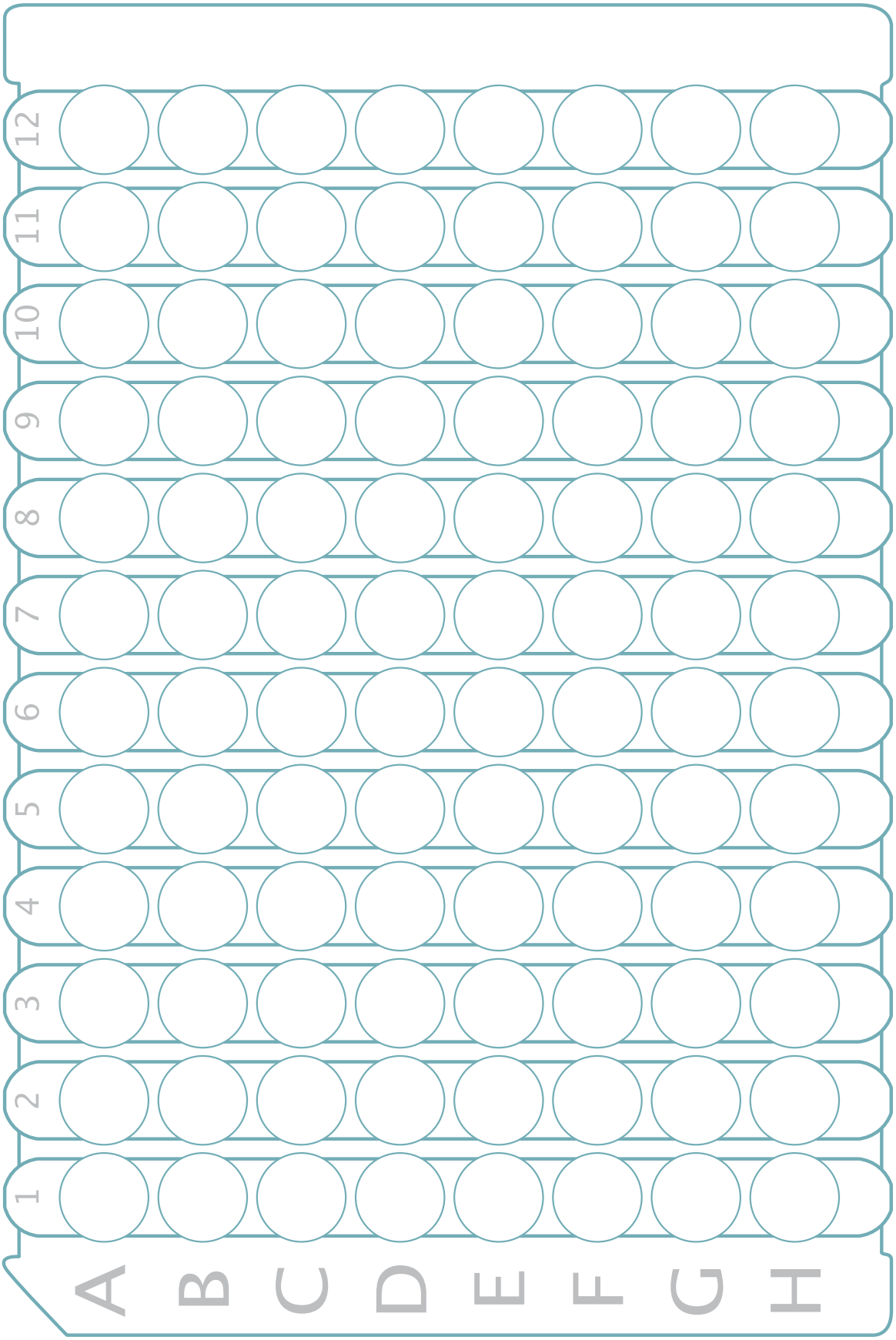
Insulin

REFERENCES

1. Blundell, T.L. and R.E. Humbel (1980) Nature **287**:781.
2. Grimberg, A. and P. Cohen (2000) J. Cell. Physiol. **183**:1.
3. Bayes-Genis, A. *et al.* (2000) Circ. Res. **86**:125.
4. Hall, K. and V.R. Sara (1983) Vitamin. Horm. **40**:175.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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