AnshLabs

FOR PROFESSIONAL USE ONLY

Total IGF-I ELISA

IVD

AL-121

INTENDED USE

The Total IGF-I enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGF-I in serum, plasma and other biological fluids. This assay is intended for *in vitro Diagnostic Use Only*.

SUMMARY AND EXPLANATION

IGF-I, also known as somatomedin C, is a 7.6 kDa, 70 amino acid residue peptide, which mediates the actions of growth hormone (GH).[1] IGF-I is synthesized as a prohormone, a polypeptide consisting of A, C, B, D, and E domains.[1,2] After post-translational modification, the mature IGF-I consists of the A, C, B and D domains, and is structurally homologous to IGF-II and insulin. In vivo, IGF-I is secreted by the liver and several other tissues and is postulated to have mitogenic and metabolic actions at or near the sites of synthesis; this has been termed the paracrine role of IGF-I.[1] IGF-I also appears in the peripheral circulation where it circulates primarily in a high molecular weight tertiary complex with IGF-binding protein-3 (IGFBP-3) and acid-labile subunit (ALS).[2,3] A smaller proportion of IGF-I may circulate in association with other IGF-binding proteins.[3] It has been estimated that <5% of plasma IGF-I circulates unbound.[4] In vivo synthesis of IGF-I is stimulated by GH, and is also dependent on other factors, including adequate nutrition.[1,5] IGF-I may inhibit pituitary production of GH; however, a feedback mechanism has not been completely defined.

In humans, plasma IGF-I levels are low during fetal and neonatal life, increase gradually during childhood, peak during mid-puberty, and decline gradually through adult life.[1,5-7] Average plasma IGF-I levels are slightly higher in females at each age. Maternal plasma levels increase during pregnancy.[1] Plasma IGF-I levels are stabilized by the IGF-binding proteins and there is negligible diurnal variation.[5] Plasma IGF-I levels are low relative to age- and sex-related norms in GH deficiency[5-7], malnutrition[5,8] and in the syndrome of GH-receptor deficiency (Laron dwarfism).[9] Abnormally low levels of plasma IGF-I have been used as a diagnostic indicator of GH deficiency, although a significant proportion of GH-deficient children may have IGF-I levels in the normal range, and normal short children may have low IGF-I levels.[1,6,7] Plasma IGF-I levels may also be used to monitor the short- and long-term in vivo responses to GH treatment.[5] Abnormally elevated IGF-I levels in acromegaly (GH excess) may be used as a diagnostic tool and to monitor treatment.[1,5]

Assay of plasma IGF-I is complicated by the presence of IGF-binding proteins, which may sequester IGF-I in the reaction mixture.[1] Various methods have been devised to separate the IGF and IGF-binding proteins prior to assay. Size-exclusion gel chromatography in acid is considered to be optimal[1,10], but this procedure is not feasible for routine use. Acidification followed by ethanol precipitation of the IGFBP fraction[1,11] gives results which are similar to acid-chromatography. SepPak C-18 cartridges are less convenient[11] and give variable results and relatively low recovery.

The Ansh Labs Total IGF-I Assay uses an acidification and neutralization method to dissociate IGF-I from all the binding proteins. IGF-I levels are quantified in the extracted samples using a highly sensitive and specific enzyme-linked immunosorbent assay. Also, a ratio of Free IGF-I to Total IGF-I can now be measured in individual subjects using the Ansh Free IGF-I Kit AL-122 along with the Total IGF-I kit.

PRINCIPLE OF THE TEST

The Total IGF-I is a quantitative one-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to IGF-I antibody coated microtiter wells and incubated along with horseradish peroxidase labeled antibody conjugate. After a washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the antibody-HRP conjugate binds to the solid phase antibody-antigen complex. Finally, the antibody-antigen-conjugate complex bound to the well is detected by addition of enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of IGF-I in the samples and calibrators.

MATERIALS SUPPLIED

CAL-121A - CAL-121F IGF-I Calibrators A - F

Six vials, 0.5 mL, labeled A-F, containing concentrations of Human IGF-I in the range of 0 to 50ng/mL (Refer to Calibration Card for exact values), in buffer with Pro-Clean 400. Store unopened at 0 to -20°C until the expiration date. Avoid repeated freeze thaws.

***NOTE**: The calibrators are traceable to World Health Organization IGF-I preparation NIBSC code 02/254, version 6.0.*

CTR-121-I & CTR-121-II IGF-I Controls I & II

Two vials, 0.5 mL, labeled Levels I and II containing low and high IGF-I concentrations (Refer to Calibration Card for exact values) in buffer with Pro-Clean 400. Store unopened at 0 to -20°C until the expiration date. Avoid repeated freeze thaws.

PLT-121 IGF-I Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with IGF-I antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ECR-121 IGF-I Enzyme Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing HRP-conjugated IGF-I antibody in buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SPB-121-I IGF-I Sample Buffer I

One bottle, 25 mL, containing sample buffer I with a non-mercury preservative. Store unopened at 2 to 8°C until the expiration date.

SPB-121-II IGF-I Sample Buffer II

One bottle, 25 mL, containing sample buffer II with a non-mercury preservative. Store unopened at 2 to 8°C until the expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm, and 630 nm.
2. Microplate shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.

WARNINGS AND PRECAUTIONS

***For in vitro Diagnostic Use Only*.**

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[12].

WARNING: Potential Chemical Hazard

Some reagents in this kit may contain Pro-Clean 400 and Sodium azide[13] as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION

a) Serum or plasma is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples should be stored frozen at -20°C or lower.

d) Avoid repeated freezing and thawing of samples.

e) Avoid assaying lipemic, hemolyzed or icteric samples.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Total IGF-I ELISA assay. It is the customer's responsibility to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
2. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

SAMPLE PREPARATION

SAMPLE PREPARATION (1:25 dilution):

a) For each unknown sample, label one 12x75mm culture tube appropriately and add **240 µL of IGF-I Sample Buffer I.**

b) Pipette **20 µL of each sample** into the appropriate pre-labeled tubes.

c) Place the tubes in a tight-fitting tube rack and shake at a slow speed (300-400 rpm) at room temperature (23 ± 2°C) for **30 minutes**.

d) Pipette **240 µL** of **IGF-I Sample Buffer II** into each tube and shake at a slow speed (300-400 rpm) at room temperature (23 ± 2°C) for **10 minutes**.

e) Vortex well. The samples are now ready to be assayed.

***NOTE**: Calibrators and controls should NOT be treated with the Sample Buffers.*

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

1. Prepare all samples to be assayed as per the "Sample Preparation" section of this package inserts.
   NOTE: Any sample reading higher than the highest calibrator should be diluted and reassayed. *(See the "Linearity" section for dilution protocol).*
   ***NOTE**: Calibrators and controls should NOT be treated with the Sample Buffers.*
2. Label the microtitration strips to be used.
3. Pipette **50 µL** each of the **Calibrators, Controls and Unknowns** from step **1** to the appropriate wells.

4. Add **100 µL** of the **IGF-I Enzyme Conjugate-RTU** to each well using a repeater pipette.
5. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
6. Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.
7. Add **100 µL** of the **TMB chromogen solution** to each well using a repeater pipette. Avoid exposure to direct sunlight.
8. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-10 min** at room temperature (23 ± 2°C).
   ***NOTE**: Visually monitor the color development to optimize the incubation time.*
9. Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **10 minutes**, using a microplate reader set to **450 nm**.
   ***NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.*

RESULTS

***NOTE:** The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log IGF-I concentration on X-axis** using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the IGF-I concentrations in ng/mL along the x-axis, using a cubic regression or polynomial degree 3 curve-fit.
3. Determine the IGF-I concentrations of the Controls and diluted unknowns from the calibration curve by matching their mean OD readings with the corresponding IGF-I concentrations.
4. The measured concentrations of the unknown samples should be multiplied by the dilution factor (25 folds).
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. Any sample reading higher than the highest calibrator should be diluted further and reassayed. *(See the "Linearity" section for dilution protocol).* Multiply the measured concentrations in ng/mL by the appropriate dilution factor.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Total IGF-I levels in serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophilic antibodies in the samples.[14]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Each laboratory should establish internal Total IGF-I control ranges. The results should fall within established confidence limits.
- A full calibration curve, and controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Calibrators	Mean Absorbance	Conc. (ng/mL)
A1, A2	A	0.026 (Blank)	0
B1, B2	B	0.034	0.9
C1, C2	C	0.12	2.8
D1, D2	D	0.50	8.6
E1, E2	E	1.46	21.0
F1, F2	F	3.35	50.0

***CAUTION**: The above data must not be employed in lieu of data obtained by the user in the laboratory*

ANALYTICAL CHARACTERISTICS

All concentrations listed are in ng/mL.

Imprecision:

Reproducibility of the Total IGF-I assay was determined using two kit controls and one serum pool sample (n=24 for all). The study included six assays with samples CI, CII, QC1 in replicates.

Sample	Mean Conc.	Within Run		Between Run		Total	
ID	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
CI	2.087	0.086	4.14	0.100	4.81	0.132	6.35
CII	8.191	0.350	4.27	0.341	4.17	0.489	5.97
QC1	132.207	2.166	1.64	5.410	4.09	5.827	4.41

Analytical Sensitivity:

The analytical sensitivity in the Total IGF-I assay, as calculated by the interpolation of mean plus two standard deviations of 20 replicates of calibrator A (0 ng/mL) and calibrator B (0.48 ng/mL), is **0.025 ng/mL**.

Analytical Specificity:

The monoclonal antibody pair used in the assay detects IGF-I. Other related analytes at the concentration in the table below did not show any significant cross-reaction when run neat (untreated).

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	IGFBP-2	1000	ND
2	IGFBP-3	1000	0.04
3	IGFBP-4	1000	ND
4	IGFBP-5	1000	ND
5	Rat IGF-I	1000	3.16
6	IGF-I/IGFBP-3 complex	1000	0.42
7	IGF-II	1000	ND

Interference:

When potential interferents (hemoglobin, triglycerides, and bilirubin) were added at the specified concentrations to control samples, Total IGF-I concentration was within ±12% of the control as represented in the following table.

Interferents	Analyte Conc. (mg/mL)	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35	54.73	50.25	-8.177
		83.63	91.13	8.969
		125.8	115.8	-7.893
Triglycerides	5	54.73	61.15	11.74
		83.63	91.23	9.148
		125.8	132.0	4.950
Bilirubin	0.5	54.83	56.35	2.782
		87.65	92.00	4.963

		128.9	125.0	-3.044

Inhibition:

Various concentrations of IGFBP-3 were spiked into IGF-I and incubated for 30 minutes to allow for the IGF-I/IGFBP-3 complex to form. The following table outlines the results.

Sample No	IGFBP-3: IGF-I Concentration	% Recovery of IGF-I	% Inhibition of IGF-I
1	31: 1	2.4	97.6
2	15.6: 1	15.4	84.6
3	7.8: 1	63.2	36.8
4	3.1: 1	97	3

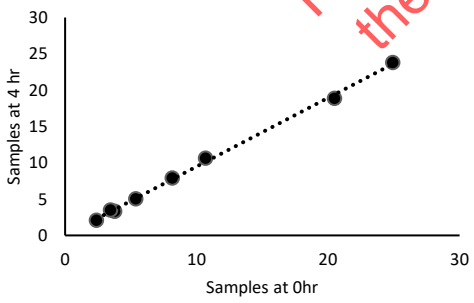
Linearity:

Three samples were used to study sample linearity. Each sample was independently treated at 1:25, 1:50 and 1:100 as per the "Sample Preparation" section. For the 1:25 dilution, 20ul of sample was added to 240ul of Sample Buffer I in the first step and then 240ul of Sample Buffer II was added in the second step. For the 1:50 dilution, 10ul of sample was added to 245ul of Sample Buffer I in the first step and then 245ul of Sample Buffer II was added in the second step. For the 1:100 dilution, 10ul of sample was added to 495ul of Sample Buffer I in the first step and then 495ul of Sample Buffer II was added in the second step. The % recovery on the individual dilutions is represented in the following table:

Sample	Treatment Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1:25	8.153	NA	NA
	1:50	4.077	4.142	102%
	1:100	2.038	1.878	92%
2	1:25	20.483	NA	NA
	1:50	10.242	9.222	90%
	1:100	5.121	4.504	88%
3	1:25	10.686	NA	NA
	1:50	5.343	4.446	83%
	1:100	2.672	2.476	93%

Treated Sample Stability:

Sample stability has been studied with eight samples prepared as per the "Sample Preparation" section. These eight samples were assayed at 0 hour and 4-hour intervals after preparation. Regression analysis yielded the following equation: **4hr** = 0.95(**0hr**) + 0.02 (R^2 = 0.99)



Sample Type:

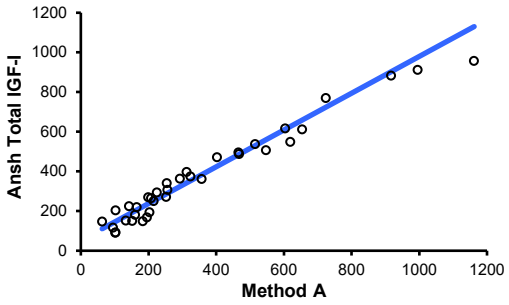
Fifty-two matched Serum, Lithium heparin plasma and Potassium EDTA plasma specimens in the range of 26-400 ng/mL were compared in the Ansh Total IGF-I assay.

Passing Bablok analysis of the results yielded the following regressions:

1. Lithium Heparin Plasma = 0.87(Serum) - 5.36, (r=0.85; P<0.0001)
2. Potassium EDTA Plasma = 0.86(Serum) - 5.61, (r=0.85; P<0.0001)
3. Potassium EDTA Plasma = 1.00(Lithium hep)- 0.45, (r=0.87; P<0.0001)

Method Comparison:

The Ansh Total IGF-I ELISA has been compared to Mediagnost IGF-I ELISA (E20) assay (Method A) using 36 samples in the range of 63 ng/mL to 1162 ng/mL. Passing Bablok analysis of the results yielded the following Regression: Total IGF-I ELISA (AL-121) = 0.93 (Method A) + 52.21 (r=0.97; P<0.0001).



The Ansh Total IGF-I ELISA has also been compared to IDS-iSYS IGF-I assay (Method B) using 45 samples in the range of 32.3 ng/mL to 265.0 ng/mL. Passing Bablok analysis of the results yielded the following Regression: Total IGF-I ELISA (AL-121) = 1.81 (Method B) – 26.54 (r=0.957).

Expected Values:

Male and Female serum samples were analyzed using Ansh Total IGF-I ELISA. The expected ranges were calculated on these samples using 95% non-parametric estimation using Analyse-It® for Microsoft Excel.

Sample	Number of specimens	Median Age	Median Total IGF-I (ng/mL)	Total IGF-I Reference Range (ng/mL)
Females (12-25yrs)	12	18	463.76	118.76 – 822.13
Females (26-50yrs)	35	32	209.18	48.75 – 407.37
Females (51-90yrs)	32	66	189.25	46.24 – 317.17
Males (2-25yrs)	13	17	355.83	231.32 – 406.70
Males (26-50yrs)	38	37	244.73	105.40 – 400.90
Males (51-90yrs)	26	67	203.21	68.00 – 325.50

The expected ranges for Total IGF-I in pediatric male samples in the age range of 3.0 – 18.0 years were calculated using 95% non-parametric estimation. A total of 404 samples in Pubic Hair Tanner stages 1 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Pubic Hair Tanner Stage	Number of specimens (n)	Median Conc. (ng/mL)	Total IGF-I (ng/mL) 95% CI
1	218	225.3	91.2 - 558.8
2	54	387.9	149.3 - 809.5
3	32	650.9	299.5 - 1053.9
4	50	713.3	430.2 - 1051.6
5	50	636.6	389.5 - 1001.8

The expected ranges for Total IGF-I in pediatric female samples in the age range of 2.4 – 18.0 years were calculated using 95% non-parametric estimation. A total of 432 samples in Breast Tanner stages 0 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Breast Tanner Stage	Number of specimens (n)	Median Conc. (ng/mL)	Total IGF-I (ng/mL) 95% CI
0	15	186.7	86.4 - 519.8
1	174	266.0	106.2 - 534.2
2	61	384.2	195.3 - 1022.6
3	58	817.3	477.0 - 1089.6
4	53	711.2	348.5 - 1135.7
5	71	619.5	270.1 - 979.6

NOTE: *It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.*

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This assay is intended for *in-vitro Diagnostic Use Only*.

Manufactured by: