IGF-II ELISA

AL-131

RUO

INTENDED USE

The Insulin-Like Growth Factor II (IGF-II) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGF-II in EDTA Plasma, serum and other biological fluids. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Insulin-like growth factor II (IGF-II) is a 7.5 kDa, 67 amino acid peptide, which is thought to mediate some of the actions of growth hormone (GH).[1] IGF-II 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-II consists of the A, C, B and D domains, and is structurally homologous to IGF-I and proinsulin. Significant quantities of pro-IGF-II containing the E-peptide extension may also be secreted into the circulation.

In vivo, IGF-II is secreted by the liver and 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-II.[1] IGF-II 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.[3,4] A smaller proportion of IGF-II may circulate in association with other IGF-binding proteins.[4] The proportion of unbound IGF-II in the circulation has been estimated at < 5%.[5] Plasma levels of IGF-II are dependent upon adequate levels of GH and other factors, including adequate nutrition.[1,6] The actions of IGF-II are mediated by binding to specific cell surface receptors. The IGF-II or type II IGF receptor is a monomeric protein which also serves as the receptor for mannose-6-phosphate.[7] The function of the type II IGF receptor is not completely defined. IGF-II binds with lower affinity to the IGF-I type I receptors and the insulin receptors. These latter receptors may mediate the mitogenic and metabolic actions of IGF-II.[8,9] Although its specific physiologic role has not been defined, it has been postulated that the interplay of IGF-I and IGF-II with the different cell surface receptors and circulating binding proteins modulates tissue growth.[9]

In humans and rodents, IGF-II messenger RNA expression is highest during fetal life.[9] Type II IGF receptor levels also appear to be high during fetal life, both in fetal tissues and in the circulation.[9,10,11] However, fetal plasma levels of IGF-II are relatively low in humans and increase with postnatal age, whereas fetal levels are higher than postnatal levels in other species.[9] Postnatal plasma IGF-II levels are assumed to be at maximal levels, since administration of GH does not result in increased IGF-II levels (unlike IGF- I levels, which increase).[12] Postnatal plasma IGF-II levels show a moderate age- related increase throughout childhood and puberty, and there is no significant variability during the day.[6,13]

The assay of plasma IGF-II is complicated by the presence of IGF-binding proteins, which may sequester IGF-II in the reaction mixture.[6,12] Various methods have been devised to separate the IGF-II and IGF-binding proteins prior to assay. Size-exclusion gel chromatography in acid is considered to be optimal,[14] but this procedure is not feasible for routine use. Acidification followed by ethanol or acetone precipitation of the IGF fraction gives results which are similar to acid-chromatography.[15]

PRINCIPLE OF THE TEST

The IGF-II ELISA assay is a quantitative two-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to IGF-II antibody coated microtiter wells and incubated. After first incubation and washing step, the wells are incubated with horseradish peroxidase labelled antibody conjugate. After a second incubation and 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. This antibody-antigen and 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-II in the samples and calibrators.

MATERIALS SUPPLIED

CAL-131A - CAL-131F   IGF-II Calibrators A thru F (Lyophilized)
Six vials, labeled A-F, containing concentrations of approximately 20-1200 ng/mL IGF-II in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened vial at 2 to 8°C until the expiration date. Reconstitute calibrators A-F with 1.0 mL deionized water. Solubilize for 10 minutes, Mix well and use after reconstitution. Aliquot and Freeze in plastic vials for multiple use. Alternatively, freeze in the same vial within 2 hours of reconstitution. Avoid repeated freeze thaws.
***NOTE**: The calibrators are traceable to World Health Organization IGF-II preparation NIBSC code 96/538, version 4.0.*

CTR-131-I & CTR-131-II   IGF-II Controls I & II (Lyophilized)
Two vials, labeled Levels I and II containing low and high IGF-II concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1.0 mL deionized water. Solubilize for 10 minutes, Mix well and use after reconstitution. Aliquot and Freeze in plastic vials for multiple use. Alternatively, freeze in the same vial within 2 hours of reconstitution. Avoid repeated freeze thaws.

PLT-131   IGF-II Coated Microtitration strips
One stripholder, containing 12 strips and 96 microtitration wells with IGF-II 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.

ASB-131   IGF-II Assay Buffer
One bottle, 12 mL, containing a protein-based (BSA) buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

ECR-131   IGF-II Enzyme Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing HRP-conjugated IGF-II antibody in buffer with a non-mercury preservative. Store at 2-8°C until 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. Repeater Pipette.
6. Vortex mixer.
7. Deionized water.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

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.[16]

WARNING: Potential Chemical Hazard

Some reagents in this kit may contain Pro-Clean 400 and Sodium azide[17] 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) EDTA Plasma and Serum are the recommended sample types.
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 IGF-II 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. **IGF-II Calibrators A-F and IGF-II Controls I & II**: Tap and reconstitute IGF-II Calibrators A-F and IGF-II Controls I & II each with **1.0 mL** deionized water. Solubilize for 10 minutes, mix well and use after reconstitution.
   NOTE: In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.
   Calibrator B/2: Mix 100 µL of reconstituted Calibrator B with 100 µL of Calibrator A
2. **Wash Solution**: Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
3. **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.

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.

NOTE:

a) Any sample reading higher than the highest calibrator should be diluted into a low reading sample and reassayed.

b) In case sensitivity below calibrator B level is desired, run reconstituted calibrator B as B/2 in the calibration curve.

1. Label the microtitration strips to be used.
2. Pipette **25 µL** each of the **Calibrators, Controls and Unknowns** to the appropriate wells.
3. Add **100 µL** of the **IGF-II Assay Buffer** to each well using a repeater pipette.
4. 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).

5. Aspirate and wash each strip **5 times** with Wash Solution **(350 μL/per well)** using an automatic microplate washer.

6. Add **100 μL** of the **IGF-II Enzyme Conjugate-RTU** to each well using a repeater pipette.

7. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).

8. Aspirate and wash each strip **5 times** with Wash Solution **(350 μL/per well)** using an automatic microplate washer.

9. Add **100 μL** of the **TMB chromogen solution** to each well using a repeater pipette. Avoid exposure to direct sunlight.

10. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    NOTE: *Visually monitor the color development to optimize the incubation time.*

11. 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-II 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. Optimum results can be obtained at incubation temperature of **(23 ± 2°C)**.

2. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.

3. Plot the **log** of the mean OD readings for each of the Calibrators along the y-axis versus **log** of the IGF-II concentrations in ng/mL along the x-axis, using a cubic regression or polynomial degree 3 curve-fit.

4. Determine the IGF-II concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding IGF-II concentrations.

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 into a low reading sample and reassayed. For best results, dilute samples no more than eight folds. Multiply the result with the appropriate dilution factor.*

LIMITATIONS

The reagents supplied in this kit are optimized to measure IGF-II 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.[18]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Each laboratory should establish internal IGF-II control ranges. The results should fall within established confidence limits.
- The confidence limits for IGF-II controls are printed on the **Calibration card**.
- A full calibration curve, low and high level 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	0.029 (Blank)	0
B1, B2	B	0.074	20
C1, C2	C	0.218	59
D1, D2	D	0.705	203
E1, E2	E	1.599	522
F1, F2	F	3.175	1239

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.

Analytical Sensitivity:

The analytical sensitivity in the IGF-II assay, as calculated by the interpolation of mean plus two standard deviations of 20 replicates of calibrator A (0 ng/mL) and calibrator B (20 ng/mL), is **1.328 ng/mL**.

Analytical Specificity:

The monoclonal antibody pair used in the assay detects IGF-II. Other related analytes at the concentration in the table below did not show any significant cross-reaction.

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	IGFBP-2	1000	ND
2	IGFBP-3	1000	ND
3	IGFBP-4	1000	ND
4	IGFBP-5	1000	ND
5	IGF-I	1000	ND
6	IGF-I/IGFBP-3 Complex	1000	ND
7	IGF-I	1000	ND

NOTE: ND = Non-Detectable

Linearity:

Three serum samples were serially diluted into low reading Human Serum and their % recovery was calculated.

Sample	Dilution Factor	Expected Conc. (ng/mL)*	Observed Conc. (ng/mL)	% Recovery
1	Neat	NA	403.097	NA
	1:2	201.549	212.296	105
	1:4	100.774	99.292	99
2	Neat	NA	492.732	NA
	1:2	246.366	247.710	101
	1:4	123.183	114.289	93
3	Neat	NA	532.279	NA
	1:2	266.140	281.755	106
	1:4	133.070	151.853	114

Imprecision:

Reproducibility of the IGF-II assay was determined using two kit controls (n=12) and two samples (n=18) over six assay runs.

Sample	Mean Conc.	Within Run		Between Run		Total	
ID	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
CI	107.08	4.37	4.08	0.50	0.47	4.40	4.11
CII	261.90	4.62	1.76	6.36	2.43	7.86	3.00
S1	323.51	10.71	3.31	14.00	4.33	17.62	5.45

|							
S2	282.05	13.08	4.64	7.77	2.75	15.21	5.39

Interference:

Potential interferents (hemoglobin, triglycerides and bilirubin) were added at the specified concentrations to control serum samples. Results are shown in the table below.

| Interferents	Analyte Conc. (mg/mL)	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35	350.764	297.421	-15.208
		347.276	269.784	-22.314
		378.251	306.881	-18.868
		397.904	385.996	-2.993
Triglycerides	5	350.764	362.791	3.429
		347.276	343.184	-1.178
		378.251	363.95	-3.781
		397.904	443.607	11.486
Bilirubin	0.6	359.813	352.75	-1.963
		360.092	332.414	-7.917
		387.563	372.326	-3.931
		418.708	376.798	-10.008

Sample Storage Stability:

Sample storage stability (n=12) was compared between aliquots stored at room temperature, -80°C, -20°C and 2-8°C after 13 days. Results are outlined below.
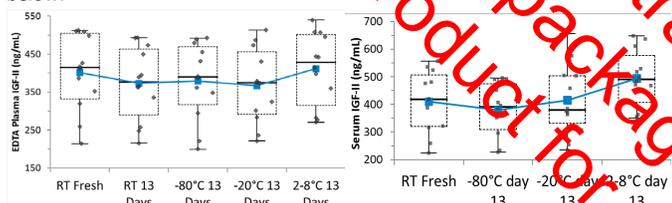
Sample Freeze/Thaw Stability:

Matched serum, EDTA plasma and Lithium Heparin plasma samples (n=10) were individually frozen/thawed 1x, 2x and 3x. Results are outlined below.
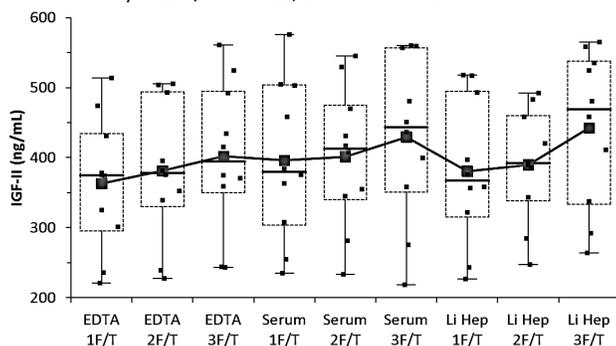
Method Comparison:

The Ansh IGF-II ELISA has been compared to a Commercial IGF-II assay (Method A) using 28 serum samples in the range of 261 ng/mL to 750 ng/mL. Passing Bablok analysis of the results yielded the following Regression: Ansh IGF-II ELISA (AL-131) = 1.10 (Method A) – 43.31 (r=0.72; P<0.0001).

REFERENCES

1. Daughaday WH, Rotwein P: Insulin like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. Endocrin Rev 10:68-91, 1989.
2. Bell GI, Merryweather JP, Sanchez-Pescador R, Stempien MM, Priestley L, Scott J, Rall LB: Sequence of a cDNA clone encoding human preproinsulin- like growth factor II. Nature 310:775-777, 1984.
3. Baxter RC, Martin JL, Beniac VA: High molecular weight insulin-like growth factor binding protein complex. J Biol Chem 264:11843-11848, 1989.
4. Rechler M: Insulin-like growth factor binding proteins. Vit Horm 47:1-114, 1993.
5. Zapf J, Hauri C, Waldvogel M, Froesch ER: Acute metabolic effects and half-lives of intravenously administered insulin-like growth factors I and II in normal and hypophysectomized rats. J Clin Invest 77:1768-1775, 1986.
6. Lee PDK, Rosenfeld RG: Clinical utility of insulin-like growth factor assays. Pediatrician 14:154-161, 1987.
7. Morgan DO, Edman JC, Standring DN, Fried VA, Smith MC, Roth RA, Rutter WJ: Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 329:301-307, 1987.
8. Conover CA, Misra P, Hintz RL, Rosenfeld RG: Effect of an anti-insulin-like growth factor I receptor antibody on insulin-like growth factor II stimulation of DNA synthesis in human fibroblasts. Biochem Biophys Res Commun 139:501-508, 1986.
9. Gluckman PD, Ambler GR: What is the function of circulating insulin-like growth factor-2 in postnatal life. Mol Cell Endocrinol 92:C1-C3, 1993.
10. Lee PDK, Hintz RL, Rosenfeld RG, Benitz WE: Presence of insulin growth factor receptors and lack of insulin receptors on fetal bovine aortic smooth muscle cells. In Vitro Cell Dev Biol 24:921-926, 1988.
11. Kiess W, Greenstein LA, White RM, Lee L, Rechler MM, Nissley SP: Type II insulin-like growth factor receptor is present in rat serum. Proc Nat Acad Sci USA 84:7720-7724, 1987.
12. Hintz RL: The somatomedins. Adv Pediatr 28:293-317, 1981.
13. Rosenfeld RG, Wilson DM, Lee PDK, Hintz RL: lnsulin-like growth factors I and II in evaluation of growth retardation. J Pediatr 109:428-433, 1986.
14. Soliman AT, Hassan AEH1, Aref MK, Hintz RL, Rosenfeld RG, Rogol AD: Serum insulin-like growth factors I and IT concentrations and growth hormone and insulin responses to arginine infusion in children with protein-calorie malnutrition before and after nutritional rehabilitation. Pediatr Res 20:1122-1130, 1986.
15. Powell DR, Rosenfeld RG, Baker BK, Liu F, Hintz RL: Serum somatomedin levels in adults with chronic renal failure: the importance of measuring insulin-like growth factor I (IGF-I) and IGF-II in acid-chromatographed uremic serum. J Clin Endocrinol Metab 63:1186-1192, 1986.
16. HHS Publication, 5th ed., 2007 Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
17. DHHS (NIOSH) Publication No. 76-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.
18. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

The Ansh Labs logo is a trademark of Ansh Labs.