IGFBP-2 ELISA

RUO

AL-140

INTENDED USE

The IGFBP-2 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGFBP-2 in human, rat, and mouse serum. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

IGFBP-2 is an unglycosylated polypetide of 31.3 kDa and is one of the six circulating proteins that bind insulin-like growth factor (IGF)-I and -II with high affinity. Cleavage of signal peptide from the precursor protein generates the 31 kDa mature IGFBP-2, which consists of N- and C-terminal cysteine rich regions.[1,2] Both N- and C-terminal regions of IGFBP-2 bind IGF-I and IGF-II preventing their interaction with IGF-1 receptor.[2] IGFBP-2 mainly exists in non-glycosylated and non-phosphorylated form.[3,4]

IGFBP-2 is a widely expressed protein with functions in bone and skeletal muscle development, and regulation of body growth and composition.[5] Pure congenic IGFBP2 -/- mice show gender and bone compartment-specific phenotypes.[6] IGFBP-2 overexpression mice have reduced body weight suggesting a role in postnatal growth by potentially regulating IGF-I bioavailability. [7]

IGFBP-2 levels in circulation are influenced under different metabolism and malignant states.[5] IGFBP-2 levels are high in cord serum from term infants, in contrast plasma IGFBP-2 levels in adults are lower. IGFBP-2 levels in adults are subjected to minimal daily fluctuations suggesting that postprandial changes in glucose and insulin levels do not influence IGFBP-2 levels. Compared to normal adults, IGFBP-2 levels are elevated in hypopituitary adults suggesting regulation by growth hormone.[8] Extreme physical exercise results in elevated serum IGFBP-2 levels in men and women. Circulating IGFBP-2 levels are high in anorexia nervosa patients and suppressed in obesity and Type 2 diabetes.[5] Overexpression of IGFBP-2 protects against obesity and diabetes by inhibiting adipogenesis and modulating insulin sensitivity.[9,10] In contrast to Type 2 diabetes, IGFBP-2 levels are increased in Type 1 diabetes suggesting regulation by insulin sensitivity.[11] Low postpartum IGFBP-2 levels are associated with the development of Type 2 diabetes in women with history of gestational diabetes mellitus.[12]

IGFBP-2 levels are also altered in intra-uterine growth retardation (IUGR) and small for gestational age (SGA) cases. In IUGR cord serum levels of IGFBP-2 are elevated and levels are low in pre-pubertal and pubertal SGA subjects. IGFBP-2 role in cancer progression is indicated by elevated levels in several malignancies like prostate, ovarian, breast and gastric cancer[5]. In colon cancer IGFBP-2 levels are elevated in plasma and is associated with increased risk of mortality.[13] Recently, IGFBP-2 was identified as an early-stage biomarker in invasive ductal adenocarcinoma of pancreas[14] and elevated serum IGFBP-2 levels were reported in idiopathic pulmonary fibrosis and Lupus nephritis patients.[15,16] Circulating levels of IGFBP-2 may be an important biomarker of different metabolic and malignant states.

PRINCIPLE OF THE TEST

The IGFBP-2 is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to IGFBP-2 antibody coated micro titer wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated IGFBP-2 antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by 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 IGFBP-2 in the samples and calibrators.

MATERIALS SUPPLIED

CAL-140A IGFBP-2 Calibrator A/Sample Diluent

One bottle, 10 mL, labeled IGFBP-2 Cal A/Sample Diluent, containing 0 ng/mL IGFBP-2 in a buffer and ProClin 300. Store unopened at 2-8°C until the expiration date.

CAL-140B - CAL-140F IGFBP-2 Calibrators B – F

Five vials, labeled B-F, containing concentrations of approximately 0.45-16ng/mL IGFBP-2 in a buffer. Refer to **calibration card** for exact concentrations. Store unopened at -20°C until the expiration date. Avoid repeated freeze thaws. The IGFBP-2 concentration in the IGFBP-2 calibrators is traceable to the manufacturer's working calibrators. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-140-I & CTR-140-II IGFBP-2 Controls I & II

Two vials, labeled Levels I and II containing low and high IGFBP-2 concentrations in buffer. Refer to **calibration card** for exact concentrations. Store unopened at -20°C until the expiration date. Avoid repeated freeze thaws.

PLT-140 IGFBP-2 Coated Microtitration strips

One stripholder, containing 12 strips and 96 microtitration wells with IGFBP-2 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-140 Assay Buffer

One bottle, 25 mL, containing a BSA based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-140 IGFBP-2 Biotin Conjugate Concentrate

One vial, 0.4 mL containing a solution of biotinylated antibody concentrate in a protein-based buffer with a non-mercury preservative. Dilute prior to use in IGFBP-2 Assay buffer. Store at 2-8°C until expiration date.

SAR-140 IGFBP-2 Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 11 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, 11 mL, containing 0.2M 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 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate absorbance reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 5-250 µL.
5. Vortex mixer.
6. Repeater pipette.

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

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. 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," 6th Edition, 2020.[17]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin 300 and Sodium azide[18] as a preservative. ProClin 300 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 is the recommended sample type. 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.

b) Samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

c) Avoid assaying lipemic, hemolyzed or icteric samples.

d) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

e) 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.[19]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the IGFBP-2 ELISA assay. It is the user's responsibility to validate the assay for their purpose. 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. Calibrator B/2: For increased sensitivity, mix 100 µL of Calibrator B with 100 µL of Calibrators A/Sample Diluent in an Eppendorf tube and vortex gently.
2. **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.
3. **IGFBP-2 Antibody-Biotin Conjugate Solution:** The IGFBP-2 Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of IGFBP-2 Assay buffer, according to the number of wells used. If an entire plate is to be used pipet exactly 220 µL of the Concentrate into 11 mL of the Assay buffer.
4. **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 (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate. *For increased sensitivity,* ***add calibrator B/2*** *to the designated well (see Preparation of Reagents).*

Protocol 1 – (Human samples)

Note: All Male, Female and First Trimester samples should be diluted 1:10 (10µL sample+ 90µL* IGFBP-2 Calibrator A/Sample Diluent*). Samples from Second Trimester onwards should be run neat. Do not dilute the calibrators and controls.

1. Allow the calibrators to reach the room temperature (23 ± 2°C) & mix well by gentle vortex.
2. Label the microtitration strips to be used.
3. Pipette **25 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add **100 µL** of the IGFBP-2 Assay Buffer 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 6**0 minutes** at room temperature (23 ± 2°C).
6. With **30-40 minutes** remaining of incubation time, prepare the IGFBP-2 Antibody-Biotin Conjugate Solution by diluting the IGFBP-2 Biotin Conjugate Concentrate in IGFBP-2 Assay buffer as described under the Preparation of the Reagents section of this insert.
7. Aspirate and wash each strip **5 times** with Wash Solution (350 **µL**/per well) using an automatic microplate washer.
8. Add **100 µL** of the IGFBP-2 Biotin Conjugate solution to each well using a repeater pipette.
9. 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).
10. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.
11. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. 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).
13. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.
14. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8 -10min** at room temperature (23 ± 2°C).
    NOTE: Visually monitor the color development to optimize the incubation time.
16. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.
17. **IMPORTANT:** All diluted specimens should be multiplied by the appropriate **dilution factor** for the final concentration.
    NOTE: *Zero calibrator (Cal. A) 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.*

Protocol 2 – (Rat and Mouse samples)

Note: All Rat samples should be diluted 1:2.5 (10µL sample+ 15µL* IGFBP-2 Calibrator A/Sample Diluent*). All Mouse samples should be diluted 1:5 (5µL sample+ 20µL* IGFBP-2 Calibrator A/Sample Diluent*). Rat and Mouse samples should be diluted directly onto the plate, per the protocol shown below. Do not dilute the calibrators and controls.

1. Allow the calibrators to reach the room temperature (23 ± 2°C) & mix well by gentle vortex.
2. Label the microtitration strips to be used.
3. Add **100 µL** of the **IGFBP-2 Assay Buffer** to each well using a repeater pipette.
4. Pipette **25 µL** of the **Calibrator and Controls** to the appropriate wells.
5. **For Rat samples:**
   a. Pipette **15 µL** of the **IGFBP-2 Calibrator A/Sample Diluent to** the sample designated wells.
   b. Pipette **10 µL** of the unknown rat sample to the sample diluent added wells.

   For Mouse samples:
   a. Pipette **20 µL** of the **IGFBP-2 Calibrator A/Sample Diluent** to the sample designated wells.
   b. Pipette **5 µL** of the unknown mouse sample to the sample diluent added wells.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for 6**0 minutes** at room temperature (23 ± 2°C).
7. With **30-40 minutes** remaining of incubation time, **prepare the IGFBP-2 Antibody-Biotin Conjugate Solution** by diluting the IGFBP-2 Biotin Conjugate Concentrate in IGFBP-2 Assay buffer as described under the Preparation of the Reagents section of this insert.
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 **IGFBP-2 Biotin Conjugate Solution** to each well using a repeater pipette.
10. 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).
11. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.
12. Add **100 µL** of the **Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.
13. 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).
14. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.
15. Add **100 µL** of the **TMB chromogen solution** to each well using a precision pipette. Avoid exposure to direct sunlight.
16. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8 -10min** at room temperature (23 ± 2°C).
    NOTE: Visually monitor the color development to optimize the incubation time.
17. Add **100 µL** of the **Stopping solution** to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.
18. **IMPORTANT:** All diluted specimens should be multiplied by the appropriate **dilution factor** for the final concentration.
    NOTE: *Zero calibrator (Cal. A) 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 data on a log vs. log scale using a cubic regression curve fit. 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 IGFBP-2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the IGFBP-2 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding IGFBP-2 concentrations.
4. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A / Sample Diluent) and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. All diluted specimen values should be multiplied by the dilution factor for the final concentration.

LIMITATIONS

The reagents supplied in this kit are optimized to measure IGFBP-2 levels in human 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 heterophile antibodies in the samples.[20]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- The IGFBP-2 ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for IGFBP-2 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	Well Contents Calibrators	Mean OD	Conc (ng/mL)
A1, A2	A	0.026 (Blank)	0
B1, B2	B/2	0.025	0.425
C1, C2	B	0.054	0.85
D1, D2	C	0.121	1.6
E1, E2	D	0.409	3.6
F1, F2	E	2.308	12.5
G1, G2	F	4.057	27.5

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in ng/mL.

Analytical Sensitivity:

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviations of 42 replicates of calibrator A (0 ng/mL) and 41 replicates of Calibrator B (0.45ng/mL) in 21 runs is 0.08 ng/mL.

Imprecision:

Reproducibility of the IGFBP-2 assay was determined in a study using two controls. The study included a total of 21 assays, two replicates of each per assay (n=24). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

Sample	Mean conc.	Within run		Between run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	1.38	0.037	2.71%	0.029	2.13%	0.048	3.45%
Control II	5.15	0.233	4.53%	0.188	3.65%	0.299	5.81%

Linearity:

Serum samples containing various levels of IGFBP-2 were serially diluted with Calibrator A/Sample Diluent. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
S-1	1:10	15.94		
	1:20	7.97	7.69	97%
	1:40	3.97	3.66	92%
	1:80	1.99	1.83	92%
S-2	1:10	20.0		
	1:20	10.0	10.87	109%
	1:40	5.0	5.25	105%
	1:80	2.5	2.43	97%
S-3	1:40	19.9		
	1:80	9.96	10.80	108%
	1:160	4.98	5.21	105%
	1:320	2.49	2.42	98%

Recovery:

Known amounts of IGFBP-2 were added to four serum samples containing different levels of endogenous IGFBP-2. The concentration of IGFBP-2 was determined before and after the addition of exogenous IGFBP-2 and the percent recovery was calculated.

Sample	Endogenous Conc. (ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1.30	3.67	3.59	98%
		2.88	2.75	95%
		2.25	2.28	102%
2	0.81	3.43	3.51	102%
		2.55	2.64	104%
		1.85	1.85	100%
3	1.30	8.87	8.69	98%
		6.35	5.8	91%
		4.33	3.89	90%
4	0.81	8.63	8.06	93%
		6.02	5.66	94%
		3.93	3.75	95%

Cross reactivity and analyte specificity:

The monoclonal antibody pair used in the assay detects IGFBP-2. Other closely related analytes at the concentrations specified in the table below did not show any significant cross-reaction.

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	IGF-I/IGFBP-3 complex	1000	ND
2	IGF-II	1000	ND
3	Human IGF-I	1000	ND
4	IGFBP-3	1000	ND
5	IGFBP-4	1000	ND
6	IGFBP-5	1000	ND
7	Mouse IGFBP-2	25	72.47%

8	Mouse IGFBP-3	50	ND
9	Mouse IGFBP-4	50	ND
10	Mouse IGFBP-5	50	ND
11	Mouse IGF-I	50	ND
12	Mouse IGF-II	50	ND
13	Rat IGF-I	50	ND
14	Rat IGF-II	50	ND

ND= Non-Detectable

Species Immunoreactivity:

The antibody pair used in IGFBP-2 assay detects Goat, Bovine, Canine, Equine, Feline, Ovine, Porcine, Mouse, Rat, and Vervet Monkey samples and does not detect Rabbit and Squirrel Monkey samples as represented below.

Sample#	Species	Type	O.D.	Conc. (ng/mL)
1	Rabbit	Serum	-0.001	0
2	Rabbit	Serum	0.013	0.55
3	Rabbit	Serum	0.002	<0.407
4	Goat	Serum	4.187	35.17
5	Goat	Serum	0.399	6.83
6	Goat	Serum	1.971	20.92
7	Bovine	Serum	4.16	35.02
8	Bovine	Serum	3.867	33.30
9	Bovine	Serum	0.078	2.11
10	Canine	Tissue Extract	2.672	25.82
11	Canine	Tissue Extract	0.029	1.01
12	Canine	Serum	0.287	5.40
13	Canine	Serum	4.163	35.03
14	Canine	Serum	2.733	26.22
15	Equine	Cyst Fluid	2.133 (1:50)	1104.60
16	Equine	Serum	4.047	34.36
17	Equine	Serum	4.291	35.77
18	Equine	Serum	0.299	5.56
19	Feline	Serum	4.002	34.10
20	Feline	Serum	4.229	35.41
21	Feline	Serum	4.124	34.81
22	Ovine	Serum	3.734	32.51
23	Ovine	Serum	4.235	35.45
24	Porcine	Serum	0.222	4.50
25	Porcine	Serum	0.112	2.74
26	Porcine	Serum	0.32	5.84
37	Squirrel Monkey	Serum	-0.004	0
38	Squirrel Monkey	Serum	-0.001	0
39	Vervet Monkey	Serum	2.762	26.41

Expected IGFBP-2 concentrations in male and female mouse and rat samples were calculated by evaluating 10 male and 10 female Sprague Dawley rat samples and 10 male and 9 female Swiss Webster mouse samples in Ansh Labs IGFBP-2 ELISA. IGFBP-2 mean, and median concentrations and range were calculated using Analyse-It® for Microsoft Excel and is shown in table below.

Sample	Gender	Strain	n	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)
Rat	Male	Sprague Dawley	10	3.0	3.1	2.1 - 4.5
	Female		10	9.21	7.17	4.0 - 23.7
Mouse	Male	Swiss Webster	10	19.9	1.75	0 - 110.7
	Female		9	83.76	96.16	36.7 - 125.5

Interference:

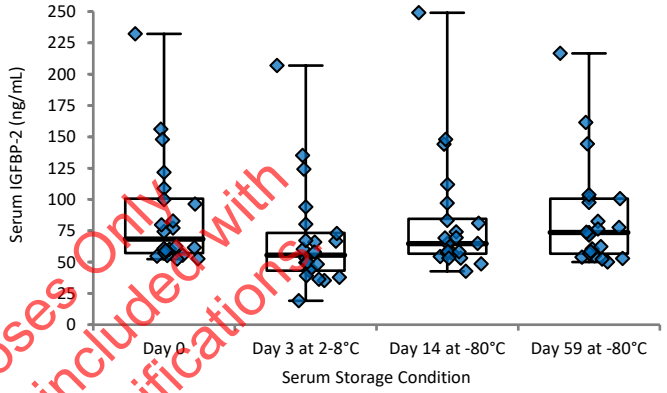
When potential interferents (hemoglobin, triglycerides, and bilirubin) were added at least at two times their physiological concentration to control sample, IGFBP-2 concentrations were within ± 10% of the control as represented in the following table.

Interferents	Analyte Conc. (mg/mL)	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35	145.22	144.47	-0.52
		205.35	203.22	-1.04
Triglycerides	5.00	145.22	147.91	1.85
		205.35	198.84	-3.17
Bilirubin	0.60	159.48	159.48	5.36
		213.48	213.48	7.14

Sample Stability:

Twenty-two serum samples were compared in the IGFBP-2 ELISA assay from day 0 (fresh draw)- day 59 for stability.

The following box plots represent serum concentrations at different time intervals and conditions of the samples.



Storage Condition	N	Median (ng/mL)	95% CI (ng/mL)
Day 0	22	68.35	57.24 - 99.79
Day 3 (2-8°C)	22	55.53	43.51 - 72.67
Day 14 (-80°C)	22	64.73	57.96 - 83.31
Day 59 (-80°C)	22	73.63	56.52 - 100.54

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