Intact Rat/Mouse IGFBP-4 ELISA RUO

AL-1025

INTENDED USE

The Intact Rat/Mouse IGFBP-4 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGFBP-4 in mouse and rat samples. 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-binding protein-4 is a member of the insulin-like growth factor binding protein (IGFBP) family and encodes a protein with an IGFBP domain and a thyroglobulin type-I domain. The cDNA for human IGFBP-4 encodes a 258-residue, for mouse IGFBP-4 encodes 254-residue proteins that is processed, by removal of the signal sequence, to a mature protein of 237 residues (25.6 kDa) with a single asparagine-linked glycosylation site[1] for human and 233 residues for mouse. Mature mouse IGFBP-4 shares 99%aa sequence homology with rat IGFBP-4 and 90.9% with human, canine, bovine, ovine and porcine IGFBP-4. Although various cell types when in culture secrete both glycosylated (28-29 kDa) and non-glycosylated (24-25 kDa) forms of IGFBP-4, the non-glycosylated is typically the most abundant in normal human blood [2, 3].

IGFBP-4 is unique among the six IGFBPs in having two extra cysteine residues in the variable L-domain and may be responsible for the distinctive biological functions of IGFBP-4 (4). Although the exact functional role for serum IGFBP-4 is not absolutely clear, in vitro studies have shown that IGFBP-4 inhibits IGF activity in bone cells and other cell types. IGFBP-4 has been reported to inhibit IGF-I- and IGF-II-induced cell proliferation of embryonic chick calvaria cells and MC3T3-E1 mouse osteoblasts [5, 6], IGF-I- and IGF-II stimulated DNA synthesis in a variety of cell types. [(3)]

Proteolysis is a major regulatory mechanism of IGFBP-4 functions. An IGF-dependent IGFBP-4-specific protease was first reported in the media conditioned by both human and sheep dermal fibroblasts. This protease was later identified as pregnancy-associated plasma protein-A (PAPP-A). It was shown that recombinant PAPP-A is an active protease able to cleave IGFBP-4 at a single site, between M135/K136. IGFBP-4 cleavage by PAPP-A is possible only in case when IGFBP is complexed with IGF. PAPP-A also cleaves IGFBP-5 between S143/K144, but in this case the presence of IGF is not required.

Several studies have shown that concentration of PAPP-A in blood of patients with acute coronary syndrome (ACS) is higher than in blood of patients with stable coronary artery disease or control subjects. PAPP-A has been suggested as a marker of cardiovascular diseases associated with coronary artery blood clotting, such as unstable angina and myocardial infarction (MI) [7-14]. It was hypothesized that in atherosclerotic plaques PAPP-A expressed by activated smooth muscles cells could function as an active enzyme cleaving IGFBP-4 complexed with IGF, thus enhancing IGF bioavailability. The IGF system might contribute to the atherosclerotic plaque development, destabilization, and rupture leading to acute coronary events [15]. It was shown that IGFBP-4 is expressed by different cells of tumor origin, such as lung adenocarcinoma, non-small-cell lung cancer, breast cancer, colon carcinoma, follicular thyroid carcinoma, gastric cancer, glioma, hepatoma, myeloma, neuroblastoma, osteosarcoma and prostate cancer. In vitro and in vivo studies suggest that IGFBP-4 plays an important role in the growth regulation of a variety of tumors, possibly by inhibiting autocrine IGF actions. Regulation of IGF bioavailability may play a crucial role in tumor growth and development [13].

The measurements of IGFBP-4 along with PAPP-A enzyme activity could be of higher clinical value than just PAPP-A measurements alone since PAPP-A concentration in blood is affected by heparin injections. The concentration of human PAPP-A, total human IGFBP-4 and intact human IGFBP-4 in human biological fluid can be measured accurately using immunoassay methods (picoPAPP-A ELISA, AL-101; Total IGFBP-4 ELISA, AL-126; and Intact IGFBP-4 ELISA, AL-128 respectively). Similarly, the concentration of mouse PAPP-A and Intactmouse IGFBP-4 in biological fluids can be measured accurately using mouse PAPP-A ELISA, AL-158; and Intact Rat/Mouse IGFBP-4 ELISA, AL-1025.

PRINCIPLE OF THE TEST

The Intact Rat/Mouse IGFBP-4 is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown samples are added to IGFBP-4 antibody coated micro titer wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated IGFBP-4 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-4 in the samples and calibrators.

MATERIALS SUPPLIED

CAL-1025A IGFBP-4 Calibrator A/Sample Diluent

One bottle, 5.0 mL, labeled IGFBP-4 Calibrator A/Sample Diluent, containing 0 ng/mL IGFBP-4 in protein-based buffer and ProClin 300. Store unopened at 2-8°C until the expiration date.

CAL-1025G IGFBP-4 Calibrator G (Lyophilized)

Two vials labeled calibrator G, containing concentrations of approximately 36 ng/mL IGFBP-4 in protein-based buffer and ProClin 300. **Refer to calibration card for exact concentration**. Store unopened at 2 to 8°C until the expiration date. **Reconstitute calibrator G with 0.5 mL deionized water**. Solubilize, mix well, and use after reconstitution. Reconstitute only one vial at a time and discard after use. Use fresh vial for new assay.

Note: *The human IGFBP-4 concentration in the calibrator is traceable to the SinoBiological Mouse IGFBP-4 Protein (Catalog number 50250-M08H, Lot number LC17MA0211).*

CTR-1025-I IGFBP-4 Control I

Two vials, labeled Control I containing IGFBP-4 in protein-based buffer and ProClin 300. **Refer to calibration card for exact concentration.** Store

unopened at 2 to 8°C until the expiration date. Reconstitute Control Level I with 0.5 mL deionized water. Solubilize, mix well, and use after reconstitution. Reconstitute only one vial at a time and discard after use. Use fresh vial for new assay.

PLT-1025 **Intact Rat/Mouse IGFBP-4 Coated Microtitration strips**

One stripholder, containing 12 strips and 96 microtitration wells with anti IGFBP-4 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-1025 **Mouse IGFBP-4 Assay Buffer**

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

BCC-1025 **Intact Rat/Mouse IGFBP-4 Biotin Conjugate Concentrate**

One vial, 0.4 mL containing biotinylated detection antibody in a protein-based buffer with a non-mercury preservative. Dilute prior to use in IGFBP-4 assay buffer. Store at 2-8°C until expiration date. Store at 2-8°C until expiration date.

SAR-1025 **Intact Rat/Mouse IGFBP-4 Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)**

One 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, 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 2-30°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, 405 nm, 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. 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

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," 5th Edition, 2007.[16]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin 300 and Sodium azide[17] 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, which can be obtained at AnshLabs.com or by request.

SAMPLE COLLECTION

a) Serum is the recommended sample type. Plasma and other biological fluids can also be used.

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

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of 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 Rat/Mouse IGFBP-4 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 components 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. **IGFBP-4 Calibrator G and Control I**: Tap and reconstitute IGFBP-4 Calibrator G and IGFBP-4 Control I each **with 0.5 mL deionized water**. Solubilize, mix well, and use after reconstitution.
2. **Preparation of Calibrators B - F**:
   a. Prepare five tubes and label them as Cal. B, Cal. C, Cal. D, Cal. E, and Cal. F.

b. Add 100 µL of Calibrator A/Sample Diluent to each tube labeled Cal. B -Cal. F.

c. **Cal. F:** Add 100 µL of reconstituted IGFBP-4 Calibrator G (from step 1) to the tube labeled Cal. F. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

d. **Cal. E:** Add 100 µL of Cal F (from step c) to the tube labeled Cal. E. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

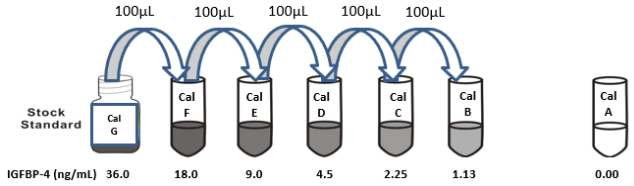
e. **Cal. D:** Add 100 µL of Cal. E (from step d) to the tube labeled Cal. D. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

f. **Cal. C:** Add 100 µL of Cal. D (from step e) to the tube labeled Cal. C. Vortex and mix the content in the tube thoroughly before use.

g. **Cal. B:** Add 100 µL of Cal. C (from step f) to the tube labeled Cal. B. Vortex and mix the content in the tube thoroughly before use.

h. In case sensitivity below calibrator B level is desired, dilute calibrator B (from step g) as below.
Cal. B/2: Mix 50 µL of Cal B (from step g) with 50 µL of calibrator A.

A schematic representation of the dilutions is shown below. Refer to the calibration card provided in the kit for exact concentration.



3. **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.
4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed back in the resealable pouch with the desiccant. The pouch must be resealed to protect it from moisture.
5. **Mouse IGFBP-4 Antibody-Biotin Conjugate Solution**: The IGFBP-4 Antibody-Biotin Conjugate Concentrate should be diluted **at a ratio of 1 part conjugate to 50 parts of IGFBP-4 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 buffer.

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. Label the microtitration strips to be used.
2. Pipette **20 µL** of the **Calibrators A, B/2, B, C, D, E, and F, and Control I** to the appropriate wells.
3. Pipette **10 µL** of samples using precision pipette to the sample designated wells.
4. Pipette **10 µL** of Calibrator A/Sample Diluent (CAL-1025A) to the sample added wells.
5. Add **100 µL** of the **Mouse IGFBP-4 Assay Buffer** to each well using a repeater pipette.
6. 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)
7. During the last **20-30 minutes** of the incubation, **prepare the Intact Rat/Mouse IGFBP-4 Antibody-Biotin Conjugate Solution** by diluting the Mouse IGFBP-4 Biotin Conjugate Concentrate (BCC-1025) in the 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 Intact Rat/Mouse **IGFBP-4 Biotin Conjugate Solution** (As specified in Preparation of Regents, step 4) 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 repeater pipette. Avoid exposure to direct sunlight.
16. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature.
NOTE: Visually monitor the color development to optimize the incubation time.
17. Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.
NOTE: *Zero calibrator should be programmed as "**Blank**" while reading the optical density. If an 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 IGFBP-4 concentration on*
X-axis using a cubic regression curve-fit. 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 mean OD readings for each of the Calibrators along the y-axis versus log of the IGFBP-4 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the IGFBP-4 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding IGFBP-4 concentrations.
5. **Multiply the sample results with the dilution factor (2X).**
6. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A) and re-assayed. Multiply the concentration by a dilution factor used.
7. Any sample reading lower than the analytical sensitivity should be reported as such.

LIMITATIONS

The reagents supplied in this kit are optimized to measure IGFBP-4 levels in rat and mouse 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.[18]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Rat/Mouse IGFBP-4 ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for IGFBP-4 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 Absorbance (OD)	Conc (ng/mL)
A1, A2	A	0.057 (Blank)	0
B1, B2	B/2	0.064	0.56
C1, C2	B	0.125	1.13
D1, D2	C	0.256	2.25
E1, E2	D	0.562	4.5
F1, F2	E	1.255	9.0
G1, G2	F	3.027	18.0

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

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in ng/mL.

Analytical Sensitivity:

The Analytical sensitivity or Minimum Detectable Dose (MDD) for Intact Rat/Mouse IGFBP-4 ELISA was calculated from six assay runs with eight replicates (n=48) of calibrator A and two replicates (n=12) as per EP10A-3 and was determined to be 0.07 ng/mL.

Imprecision:

Reproducibility of the Intact Rat/Mouse IGFBP-4 assay was determined in a study using samples in the low, mid, and high range. The study included a total of 5 assays, 3 replicates of each per assay (n=15). Representative data were calculated based on EP10A-3 guidelines and are presented in the following table.

Sample	Mean Conc. (ng/mL)	SD	%CV
1	1.3	0.065	4.94%
2	4.3	0.09	2.04%
3	12.02	0.4	3.30%

Cross reactivity and specificity:

The monoclonal antibody pair used in the assay detects IGFBP-4. Closely related analytes when tested in the assay in the concentrations shown in the table did not show any detectable cross reactivity.

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	Mouse IGFBP-2	50 ng/mL	Non-Detectable
2	Mouse IGFBP-3	50 ng/mL	Non-Detectable
3	Mouse IGFBP-5	50 ng/mL	Non-Detectable
4	Rat IGF-I	50 ng/mL	Non-Detectable
5	Rat IGF-II	50 ng/mL	Non-Detectable
6	Mouse IGF-I	50 ng/mL	Non-Detectable
7	Mouse IGF-II	50 ng/mL	Non-Detectable
8	Rec. Mouse IGFBP-4 (R&D Systems)	25 ng/mL	53.1%
9	Rec. Human IGFBP-4	18 ng/mL	100%

Species Immunoreactivity:

The antibody pair used in the assay detects Rabbit, Goat, Bovine, Canine, Equine, Feline, Ovine, and Porcine as represented in table below.

Sample#	Species	Type	O.D.	Conc. (ng/mL)
1	Rabbit	Serum	0.094	3.895
2	Rabbit	Serum	0.275	11.025
3	Rabbit	Serum	0.116	4.857
4	Goat	Serum	4.027	124.85
5	Goat	Serum	2.927	93.25
6	Goat	Serum	3.939	122.33
7	Bovine	Serum	0.235	9.56
8	Bovine	Serum	0.310	12.32
9	Bovine	Serum	0.139	5.80
10	Canine	Serum	0.033	1.25
11	Canine	Serum	1.830	61.02
12	Canine	Serum	0.032	1.16
13	Equine	Cyst Fluid	0.042 (1:50)	80.90
14	Equine	Serum	3.885	120.76
15	Equine	Serum	0.446	17.19
16	Equine	Serum	0.966	34.48
17	Feline	Serum	0.304	12.14
18	Feline	Serum	0.608	22.75
19	Ovine	Serum	1.620	54.70
20	Ovine	Serum	1.524	51.79
21	Ovine	Serum	2.078	68.40
22	Porcine	Serum	0.201	8.22
23	Porcine	Serum	0.108	4.53

Linearity:

Calibrator G and four rat samples containing various IGFBP-4 levels were serially diluted in calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Value in ng/mL	Observed Value in ng/mL	%Recovery
Cal. G	2	18.00		
	4	9.00	9.26	103%
	8	4.50	4.60	102%
	16	2.25	2.36	105%
	32	1.13	1.09	97%
	64	0.56	0.56	99%
1	4	4.04		
	8	2.02	2.21	109%
	16	1.01	1.07	106%
	32	0.51	0.42	83%
2	4	4.64		
	8	2.32	2.68	115%
	16	1.16	1.38	119%
	32	0.58	0.56	97%
3	2	7.39		
	4	3.70	3.50	95%
	8	1.85	1.69	91%
	16	0.92	0.84	90%
	32	0.46	0.38	83%
4	2	10.80		
	4	5.40	5.36	99%
	8	2.70	2.58	95%
	16	1.35	1.20	89%

Interference:

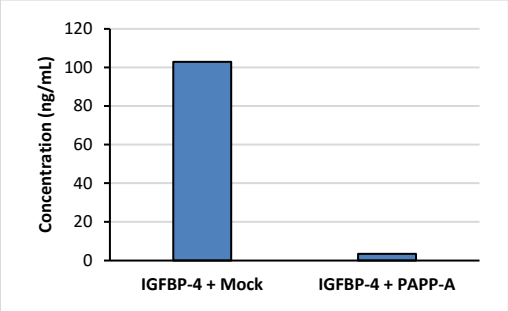
When hemoglobin, biotin, intralipids and bilirubin were added at a concentration greater than two folds of their physiological concentration to control sample, average IGFBP-4 concentrations were within ± 10% of the control as represented in the following table.

Interferent	Interferent Dose	Sample (ng/mL)	Dosed Sample (ng/mL)	% Difference
Hemoglobin	1 mg/mL	3.99	3.77	-5.5
	0.5 mg/mL	3.95	3.90	-1.2
	0.1 mg/mL	4.05	4.15	2.7
Biotin	1200 ng/mL	3.94	3.90	-0.9
	600 ng/mL	3.93	3.90	-0.8
Intralipids	20 mg/mL	3.51	3.83	9.3
	10 mg/mL	3.85	4.09	6.2
	5 mg/mL	4.23	4.36	3.0
Bilirubin	0.66 mg/mL	2.82	2.87	1.9
	0.2 mg/mL	3.64	3.69	1.6

Inhibition Study:

When IGFBP-4 was incubated with and without active PAPP-A for up to six hours and the reaction mixtures measured in Intact Rat/Mouse IGFBP-4, 100% PAPP-A cleaved IGFBP-4 fragment was observed as seen in table and figure below.

Condition	Minutes	O.D. (1:5)	Observed 1:5 Conc. (ng/mL)	Final Conc. (ng/mL)
IGFBP-4 + Mock	360	4.149	20.57	102.85
IGFBP-4 + PAPP-A	360	0.064	0.689	3.445



Expected Values:

Expected IGFBP-4 concentrations in male and female rat and mouse samples were calculated by evaluating 10 male and 10 female Sprague Dawley rat samples and 10 male and 10 female Swiss Webster mouse samples in Ansh Labs Intact Rat/Mouse IGFBP-4 ELISA. IGFBP-4 mean, and median concentrations were calculated using Analyse-It® for Microsoft Excel and is shown below.

Sample	Gender	Strain	No of specimens (n)	Mean (ng/mL)	Median (ng/mL)
Rat	Male	Sprague Dawley	10	24.64	19.41
	Female		10	32.32	37.61
Mouse	Male	Swiss Webster	10	23.01	19.66
	Female		10	21.58	22.63

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