AnshLabs

# Mouse PAPP-A ELISA

**RUO**

## AL-158

## INTENDED USE

The Mouse PAPP-A enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of PAPP-A in mouse serum, cell supernatant, 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

Pregnancy-associated plasma protein-A (PAPP-A) is a novel zinc metalloproteinase that functions in many systems outside of pregnancy. PAPP-A expression has been reported in various tissues, including endometrium, testis, kidney, bone, colon, and other adult and fetal tissues.[1-12] Abundant PAPP-A mRNA expression levels were found in visceral fat and these were 10-fold higher than in subcutaneous fat.[10] PAPP-A expression significantly increases with age in kidney, brain and gonads[10] and significantly deceases with age in bone and skeletal muscle.[10] In the thymus, PAPP-A mRNA showed a biphasic response with age.[10] Data in both humans and mice suggest a role for PAPP-A in aging and age-related diseases.[10] Expression of IGFBP-5 mRNA, a marker of insulin-like growth factor-I (IGF-I) bioactivity known to be regulated by PAPP-A, paralleled the changes in PAPP-A expression with age in kidney, bone, skeletal muscle and thymus. PAPP-A is potentially proatherosclerotic and has been proposed as a new marker of inflammation, as high serum PAPP-A levels are observed in patients with renal impairment, asthma, lung cancer, etc.[1-12] Stimulation of PAPP-A expression by intermittent PTH treatment contributes to PTH bone anabolism in mice.[9] Effect of PAPP-A on tendon structure and mechanical properties have been studied[8]. However, quantitative determination of PAPP-A in mouse tissues with age are limited due to unavailability of the mouse PAPP-A ELISA.

## PRINCIPLE OF THE TEST

The Mouse PAPP-A ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to anti-PAPP-A antibody coated micro titer wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated PAPP-A 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 PAPP-A in the samples and calibrators.

## MATERIALS SUPPLIED

**CAL-158A Mouse PAPP-A Calibrator A/Sample Diluent**

One bottle, 6 mL, labeled Mouse PAPP-A Calibrator A/Sample Diluent, containing 0 ng/mL PAPP-A in protein-based buffer with non-mercury preservative. Store at 2 to 8°C until the expiration date.

**CAL-158B - CAL-158F Mouse PAPP-A Calibrator B-F**

Five vials, 1 mL each, labeled B-F containing concentrations of approximately 0.1 - 10 ng/mL hetero-tetrameric PAPP-A (ht-PAPP-A) in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Calibrators are shipped ambient. **Store at - 20°C upon receipt until the expiration date.** Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Alternatively, freeze in the same vial within 2 hours of reconstitution. Avoid repeated freeze thaws. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias. Calibrators are shipped at ambient temperature. Store unopened at 2 to 8°C until the expiration date.

**CTR-158-I - CTR-158-II Mouse PAPP-A Controls**

Two vials, labeled Levels I and II containing low and high PAPP-A in protein-based buffer with a non-mercury preservative. Refer to **calibration card** for exact control ranges. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, 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. Controls are shipped at ambient temperature. Store unopened at 2 to 8°C until the expiration date.

**PLT-158 PAPP-A Antibody Coated Microtitration Strips**

One stripholder, containing 96 polystyrene microtitration wells with anti-PAPP-A antibody immobilized to the inside wall of each well. Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

**ASB-101 PAPP-A Assay Buffer:**

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

**CND-101 PAPP-A Conjugate Diluent**

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2 to 8°C until expiration date.

**BCC-158 Mouse PAPP-A Biotin Conjugate Concentrate**

One vial, 0.4 mL containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute 10 – 30 minutes prior to use in PAPP-A conjugate diluent. Store at 2 to 8°C until expiration date.

**SAR-158 Mouse PAPP-A Streptavidin-Enzyme Conjugate Ready-to-Use**

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

**STP-100 Stopping Solution**

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

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450nm, 405nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Repeator 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

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. [13]

### WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin 300 and Sodium azide[14] 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 AND PREPARATION

a) Serum 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 may be stored at 4°C if assayed within 24 hours; otherwise, 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. Thaw samples no more than 3 times.

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. [15]

## PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Mouse PAPP-A ELISA. It is the responsibility of the customer 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 different 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 enzyme 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. **Mouse PAPP-A Calibrators B-F and Mouse PAPP-A Controls I & II:** Tap and reconstitute Mouse PAPP-A Calibrator B-F and Mouse PAPP-A Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
   Note: In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.
   Cal. 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 when stored in a tightly sealed bottle.
3. **Mouse PAPP-A Biotin Conjugate Solution:** The mouse PAPP-A Biotin Conjugate Concentrate should be diluted at a ratio of 1 part into 50 parts of the PAPP-A conjugate diluent, according to the number of wells used. For an entire plate, pipet exactly 220 µL of the Biotin Conjugate Concentrate into 11 mL of the PAPP-A Conjugate Diluent.
   NOTE: The Biotin conjugate concentrate should be freshly diluted 10–30 minutes prior to use.
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 samples and reagents to reach room temperature. Mix reagents thoroughly by gentle inversion before use. Calibrators, controls, and samples should be assayed in duplicate.

***NOTE**: All serum samples reading higher than the highest calibrator should be thoroughly mixed and diluted in the 0 ng/mL (Calibrator A) prior to assay.*

1. Mark the microtitration strips to be used.

2. Pipet **25 µL** of the calibrators, controls and samples to the appropriate wells.
3. Add **50 µL** of the PAPP-A Assay Buffer to each well using a precision pipette.
4. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **2hrs** at room temperature (23±2°C).
5. During the last 20-30 minutes of incubation, prepare the Biotin Conjugate Solution by diluting the Mouse PAPP-A Biotin Conjugate Concentrate in PAPP-A Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
6. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
7. Add **100 µL** of the biotin conjugate solution to each well using a precision pipette.
8. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **1hr** at room temperature (23±2°C).
9. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
10. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **30 minutes** at room temperature (23±2°C).
12. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
13. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. ***Avoid direct exposure to heat and sunlight.***
14. 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.*
15. Add **100 µL** of the stopping solution to each well using a precision pipette.
16. 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 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 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 PAPP-A concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the PAPP-A concentrations of the unknowns from the calibration curve by matching their mean OD readings with the corresponding PAPP-A concentrations.
4. Any sample reading higher than the highest calibrator should be appropriately diluted using Mouse PAPP-A Calibrator A and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. If a high reading sample is diluted, multiply the value by the appropriate dilution factor.

## LIMITATIONS

The reagents supplied in this kit are optimized to measure PAPP-A levels in mouse serum and other biological fluids. 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. [16]

## QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- The confidence limits for Mouse PAPP-A ELISA controls are printed on the calibrator card.
- A full calibration curve, low- and high-level controls, should be included in each assay.
- The TMB chromogen solution should be colorless. Development of a blue color may indicate reagent contamination or instability.

## REPRESENTATIVE CALIBRATION CURVE DATA

| Well Number | Well Contents Calibrators | Mean Absorbance | Conc (ng/mL) |
|---|---|---|---|
| A1, A2 | A | 0.079 **(Blank)** | 0 |
| B1, B2 | B | 0.077 | 0.24 |
| C1, C2 | C | 0.134 | 0.39 |
| D1, D2 | D | 0.481 | 1.3 |
| E1, E2 | E | 1.563 | 3.9 |
| F1, F2 | F | 3.343 | 10.3 |

***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 ng/mL. (1 ng/ml (native htPAPP-A) = 0.00256 mIU/mL)

### Analytical Sensitivity

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 16 replicates of calibrator A (0 ng/mL) and calibrator B (0.24 ng/mL) is 0.020 ng/mL.

### Linearity

Calibrator F and mouse PAPP-A antigen were diluted with Calibrator A. The percentage recovery is represented in the following table.

| Sample | Dilution Factor | Expected Conc. (ng/mL) | Observed Conc. (ng/mL) | % Recovery |
|---|---|---|---|---|
| Calibrator-F | Neat | 10.31 | NA | NA |
| | 1:2 | 5.15 | 5.21 | 101% |
| | 1:4 | 2.58 | 2.57 | 100% |
| | 1:8 | 1.29 | 1.33 | 103% |
| | 1:16 | 0.64 | 0.68 | 105% |
| Mouse PAPP-A Antigen | Neat | 9.65 | NA | NA |
| | 1:2 | 4.83 | 4.76 | 99% |
| | 1:4 | 2.41 | 2.35 | 97% |
| | 1:8 | 1.21 | 1.18 | 98% |
| | 1:16 | 0.60 | 0.63 | 104% |

### Recovery

Mouse serum was spiked with Mouse PAPP-A Antigen at three different levels and assayed. The spike recovery is shown below.

| Sample | Endogenous Conc.(ng/mL) | Expected Conc. (ng/mL) | Observed Conc. (ng/mL) | % Recovery |
|---|---|---|---|---|
| Mouse Serum | 0.165 | 0.596 | 0.553 | 93% |
| | | 1.006 | 0.956 | 95% |
| | | 1.89 | 1.782 | 94% |

### Imprecision

Two controls and two serum sample pools were assayed in 16 replicates to determine intra-assay precision. Values obtained are shown below.

| Sample | Mean conc. (ng/mL) | Within run | | Between run | | Total | |
|---|---|---|---|---|---|---|---|
| | | SD | %CV | SD | %CV | SD | %CV |
| Control-I | 1.084 | 0.017 | 1.56 | 0.038 | 3.48 | 0.041 | 3.81 |
| Control-II | 3.042 | 0.087 | 2.84 | 0.031 | 1.02 | 0.092 | 3.02 |
| QC1 | 3.384 | 0.039 | 1.15 | 0.058 | 1.73 | 0.070 | 2.08 |
| QC2 | 3.275 | 0.074 | 2.27 | 0.059 | 1.81 | 0.095 | 2.90 |

### Analytical Specificity:

Antibody pair used in the assay is specific to PAPP-A. Other related molecules at the concentration specified in the table below did not show any significant cross-reaction.

| Cross-Reactant | Concentration | % Cross-reactivity |
|---|---|---|
| Stanniocalcin-1 | 50ng/mL | ND |
| Stanniocalcin-2 | 50ng/mL | ND |
| Alpha-2-Macroglobulin | 500ng/mL | ND |
| proMBP | 100ng/mL | ND |

### Species Immunoreactivity:

The antibody pair used in Mouse PAPP-A assay detects Goat, Bovine, Canine, Canine tissue extract, Equine, Equine cyst fluid, Feline, Ovine, Porcine, Mouse, Rat, Mouse testis extract, Squirrel Monkey, and Vervet Monkey samples and does not detect Rabbit samples as represented below.

| Sample# | Species | Type | O.D. | Conc. (ng/mL) |
|---|---|---|---|---|
| 1 | Rabbit | Serum | 0.016 | <0.048 |
| 2 | Rabbit | Serum | 0.018 | <0.048 |
| 3 | Rabbit | Serum | 0.016 | <0.048 |
| 4 | Goat | Serum | 0.202 | 0.47 |
| 5 | Goat | Serum | 0.135 | 0.316 |
| 6 | Goat | Serum | 0.103 | 0.245 |
| 7 | Bovine | Serum | 0.055 | 0.134 |
| 8 | Bovine | Serum | 0.099 | 0.236 |
| 9 | Bovine | Serum | 0.043 | 0.109 |
| 10 | Canine | Tissue Extract | 4.083 | 12.648 |
| 11 | Canine | Tissue Extract | 4.191 | 13.067 |
| 12 | Canine | Serum | 0.231 | 0.539 |
| 13 | Canine | Serum | 0.032 | 0.081 |
| 14 | Equine | Cyst Fluid | 0.181 | 21.2 |
| 15 | Equine | Serum | 0.062 | 0.152 |
| 16 | Equine | Serum | 0.048 | 0.118 |
| 17 | Equine | Serum | 0.057 | 0.138 |
| 18 | Feline | Serum | 0.034 | 0.088 |
| 19 | Feline | Serum | 0.059 | 0.145 |
| 20 | Ovine | Serum | 0.045 | 0.113 |
| 21 | Ovine | Serum | 0.377 | 0.882 |
| 22 | Porcine | Serum | 0.123 | 0.289 |
| 23 | Porcine | Serum | 0.122 | 0.286 |
| 24 | Porcine | Serum | 0.123 | 0.289 |
| 25 | Mouse | Serum | 0.252 | 0.586 |
| 26 | Mouse | Serum | 0.03 | 0.78 |
| 27 | Mouse | Serum | 0.027 | 0.69 |
| 28 | Mouse | Serum | 0.025 | 0.67 |
| 29 | Mouse, Swiss Webster | Serum | 0.083 | 0.232 |
| 30 | Mouse, Swiss Webster | Serum | 0.056 | 0.157 |
| 31 | Mouse, Swiss Webster | Serum | 0.064 | 0.18 |
| 32 | Mouse | Testis Extract | 2.806 | 9.902 |
| 33 | Mouse | Testis Extract | 1.094 | 3.03 |
| 34 | Mouse | Testis Extract | 1.038 | 2.859 |
| 35 | Rat | Serum | 0.026 | 0.67 |
| 36 | Rat | Serum | 0.018 | 0.5 |
| 37 | Rat | Serum | 0.019 | 0.52 |
| 38 | Rat, Sprague Dawley | Serum | 0.244 | 0.655 |
| 39 | Rat, Sprague Dawley | Serum | 0.207 | 0.559 |
| 40 | Rat, Sprague Dawley | Serum | 0.051 | 0.143 |
| 41 | Squirrel Monkey | Serum | 0.09 | 0.216 |
| 42 | Squirrel Monkey | Serum | 0.037 | 0.095 |
| 43 | Vervet Monkey | Serum | 0.038 | 0.097 |

### Expected Values:

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

| Sample | Gender | Strain | n | Mean (ng/mL) | Median (ng/mL) | Range (ng/mL) |
|---|---|---|---|---|---|---|
| Mouse | Male | Swiss Webster | 10 | 0.169 | 0.150 | 0.124 - 0.285 |
| | Female | | 10 | 0.15 | 0.12 | 0.071 - 0.426 |
| Rat | Male | Sprague Dawley | 10 | 0.259 | 0.184 | 0.068 - 0.655 |
| | Female | | 10 | 0.14 | 0.14 | 0.062 - 0.191 |



### REFERENCES

1. Bulut I, Coskun A, Ciftci A et al. Relationship between Pregnancy associated plasma protein A and lung cancer. The American Journal of the Medical sciences. April 2009; 337 (4): 241-244
2. Schindler AM, Bischof B. Histochemical localization of pregnancy associated plasma protein-A in fetal, infant, and adult organs and comparison between antisera. Gynecol Obstet Invest. 1984; 18: 88-94.
3. Overgaard MT, Oxvig C, Christiansen M, et al. Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the preform of eosinophil major basic protein: expression in human reproductive and nonreproductive tissue. Biol Reprod 1999; 61:1083-1089.
4. Fialova L, Kalousova M, Soukupova J, et al. Relationship of pregnancy associated plasma protein A to renal function and dialysis modalities. Kidney Blood Press Res. 2004; 27: 88-95.
5. Coskun A, Balbay O, Duran S et al. Pregnancy-associated plasma protein A and asthama. Adv Ther. 2007; 24: 362-367.
6. Coskun A, Duran S, Apaydin S, et al. Pregnancy-associated plasma protein A: evaluation of a new biomarker in renal transplanted patients. Transplant Proc. 2007; 39:3072-3076.
7. Bayes-Genis A, Conover CA, Overgaard MT, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med. 2001; 345: 1022-1029.
8. Yang TH, et al. PAPP-A affects tendon structure and mechanical properties. J Struct Biol. Oct 2015; 192(1):59-66.
9. Clifton KB, et al. Pregnancy-associated plasma protein-A modulates the anabolic effects of parathyroid hormone in mouse bone. Bone. Dec 2015; 81:413-6.
10. Harstad SL, et al. Tissue-specific changes in pregnancy associated plasma protein-A expression with age in mice. Endocrinology. Sep 2014; 57:13-7.
11. Bale LK, et al. Inducible reduction in pregnancy-associated plasma protein-A gene expression inhibits established atherosclerotic plaque progression in mice. Endocrinology, Apr 2014; 155(4):1184-7.
12. Conover CA, et al. Preferential impact of pregnancy-associated plasma protein-A deficiency on visceral fat in mice on high-fat diet. Am J Physiol Endocrinol Metab. Nov 2013; 305(9): E1145-53.

13. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
14. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.
15. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
16. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

**FOR RESEARCH USE ONLY**

Not for use in diagnostic procedures.



Manufactured by:
Ansh Lab.
445 Medical Center Blvd.
Webster, TX 77598-4217 U.S.A.