PAPP-A ELISA

RUO

AL-106

INTENDED USE

The PAPP-A enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of PAPP-A in 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

Maternal serum concentrations of pregnancy-associated plasma protein A (PAPP-A, EC 3.4.24.79, papalysin-1) are used to predict occurrence of Down syndrome. During pregnancy, PAPP-A is produced in high concentrations by the trophoblast and released into maternal circulation. In pregnancy, PAPP-A primarily circulates as 500-kDa heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (proMBP), which inhibits the proteolytic activity of PAPP-A. Dimeric PAPP-A is the only active form and proteolyses IGFBP-4 and IGFBP-5. Significant amounts of active PAPP-A is reported at gestational ages between seven and thirteen weeks.

PRINCIPLE OF THE TEST

The PAPP-A ELISA is a quantitative two-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown diluted samples are added to anti-PAPP-A 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. Finally, the 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 PAPP-A in the samples and calibrators.

MATERIALS SUPPLIED

CAL-106A - CAL-106F PAPP-A Calibrators A-F

Six vials, 0.5 mL each, labeled A-F containing concentrations of 0, 37, 150, 562, 2250 and 4500 ng/mL PAPP-A in protein based buffer with non-mercury preservative. Refer to vial labels for exact concentrations. Each calibrator concentration has been corrected for the dilution factor. Calibrators are shipped ambient. **Store at - 20°C upon receipt** until the expiration date.
The PAPP-A concentration in the AnshLite™ PAPP-A calibrators is traceable to the manufacturer's working calibrators. 1 ng/mL of purified hetero-tetrameric PAPP-A (ht-PAPP-A) characterized by amino acid analysis in AnshLite™ PAPP-A assay yields 2.56 µIU/mL. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-106-I and CTR-106-II PAPP-A Controls

Two vials, 0.5 mL each, labeled Levels I and II containing low and high concentrations of PAPP-A in protein based buffer with a non-mercury preservative. Refer to vial labels for exact control ranges. Each control concentration has been corrected for the dilution factor. Controls are shipped ambient. **Store at - 20°C upon receipt** until the expiration date.

PLT-101 Anti-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.

ECC-106 PAPP-A Antibody-Enzyme Conjugate Concentrate

One vial, 0.4 mL, containing anti-PAPP-A antibody conjugated to HRP in a protein buffer with a non-mercury preservative. Store at 2 to 8°C until the expiration date.

EXB-100 Extraction Buffer

One bottle, 50 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2 to 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 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, 11 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

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

WARNINGS AND PRECAUTIONS

For *Research Use Only*.

The following precautions should be observed:

a) Follow good laboratory practice.

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

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

WARNING: Potential Biohazardous Material

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[1].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400 and peroxide 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. Use the following recommendations for handling, processing and storing blood samples.[2]
c. Allow samples to clot for two hours at room temperature or overnight at 4°C and follow blood collection tube manufacturer's recommendations for centrifugation. Keep tubes stoppered at all times. Within two hours after centrifugation, transfer at least 500 µL of cell free sample to a storage tube. Tightly stopper the tube immediately.
    - Samples if used within 24 hours may be stored at 4°C; otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
    - Remove residual fibrin and cellular matter prior to analysis.
d. Avoid assaying lipemic, hemolyzed or icteric samples
e. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.
f. Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the PAPP-A ELISA assay. 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 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 accurately and efficiently to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
2. **PAPP-A Antibody-Enzyme Conjugate Solution:** The PAPP-A Antibody-Enzyme 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 Antibody-Enzyme Conjugate Concentrate into 11 mL of the PAPP-A Conjugate Diluent.
   NOTE: *The antibody-enzyme conjugate concentrate should be freshly diluted 10–15 minutes prior to use.*
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.

SAMPLE PREPARATION

Dilution of PAPP-A serum specimens should be performed on the same day prior to testing.

1. For each unknown serum sample, label one 12 X 75 culture tubes.
2. Add **1.05 mL** of the Extraction Buffer to each tube.
3. Add **7 uL** of the serum specimens to the pre-labeled tube and vortex well.
4. Place the tubes in a tight fitting tube rack and incubate the tubes, shaking at a **slow speed (300-400 rpm)** at room temperature for **5-10 minutes**.
5. The sample is now ready for analysis.

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature. Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipet **50 µL** of the calibrators, controls and unknown diluted samples (as described in sample preparation) 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 **2 hours** at room temperature.
5. Prepare the enzyme conjugate solution by diluting the antibody-enzyme conjugate concentrate with the PAPP-A conjugate diluent as described under the "Preparation of 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 antibody-enzyme conjugate solution to each well using a precision pipette.
8. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **1 hour** at room temperature.
9. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
10. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid direct exposure to heat and sunlight.
11. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **8-10 min** at room temperature.
    NOTE: Visually monitor the color development to optimize the incubation time.
12. Add **100 µL** of the stopping solution to each well using a precision pipette.

13. 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

1. Calculate the mean OD for each calibrator, control or diluted sample.
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 on the x-axis, using a cubic regression curve fit. For reporting the PAPP-A concentrations in mIU/mL use the following conversion factor:
   1 ng/ml (native htPAPP-A) = 0.00256 mIU/mL
3. Determine the PAPP-A concentrations of the controls and samples 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 Extraction Buffer and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.

LIMITATIONS

The reagents supplied in this kit are optimized to measure PAPP-A 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[3].

QUALITY CONTROL

a. Each laboratory should establish mean values and acceptable ranges to assure proper performance.
b. PAPP-A ELISA controls or other commercial controls should fall within established confidence limits.
c. The confidence limits for PAPP-A ELISA liquid controls are printed on the control vial labels.
d. A full calibration curve, low and high level controls, should be included in each assay.
e. 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	Mean Absorbance	Conc (ng/mL)
	Calibrators	0.011	
A1, A2	A	(**Blank**)	0
B1, B2	B	0.036	37
C1, C2	C	0.121	150
D1, D2	D	0.495	562
E1, E2	E	2.012	2250
F1, F2	F	3.686	4500

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.

Conversion Factor

Conversion factor: 1 ng/mL of htPAPP-A = 2.56 µIU/mL

Limit of Detection (LoD):

The lowest amount of PAPP-A in a sample that can be detected with a 95% probability (n=24) is 10.1 ng/mL. The value was determined by processing four serum samples in the range of 36 to 384 ng/mL following CLSI EP17 guidelines. Four assay runs per day were performed over three days with all samples run in duplicate per run.

Limit of Quantitation (LoQ):

The estimated minimum dose achieved at 7.5% total imprecision is 36 ng/mL. The value was determined by processing seven samples in the range of 36.6-2242.2 ng/mL over twelve runs and three days in duplicates (n=24) following CLSI EP17 guidelines.

Imprecision:

Reproducibility of the PAPP-A ELISA assay was determined in a study using three serum pools. The study included a total of 12 assays, four replicates of each per assay (n=48). 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
Pool-1	369.806	8.356	2.26%	4.535	1.23%	9.508	2.57%
Pool-2	505.608	8.087	1.60%	14.478	2.86%	16.583	3.28%
Pool-3	1184.171	31.686	2.68%	26.569	2.24%	41.351	3.49%

Linearity:

Based on NCCLS EP-6-P multiple dilutions of the four serum samples containing various PAPP-A levels were diluted with extraction buffer. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
	1:50	2249.504	N/A	N/A
	1:100	1124.752	1130.434	101
1	1:200	562.376	561.929	100
	1:400	281.188	301.084	107
	1:800	140.594	150.746	107
	1:50	2155.728	N/A	N/A
	1:100	1077.864	1053.054	98
2	1:200	538.932	556.500	103
	1:400	269.466	287.447	107
	1:800	134.733	139.096	103
	1:50	1655.186	N/A	N/A
	1:100	827.593	865.831	105
3	1:200	413.797	445.674	108
	1:400	206.898	227.695	110
	1:800	103.449	113.062	109
	1:50	2818.381	N/A	N/A
	1:100	1409.191	1423.607	101
4	1:200	704.595	767.728	109
	1:400	352.298	388.745	110
	1:800	176.149	191.172	109

Analytical Specificity:

The antibody pair used in the PAPP-A ELISA measures dimeric recombinant PAPP-A and PAPP-A/proMBP complex approximately in equimolar concentration and does not cross-react with PAPP-A2, MMP-9 and proMBP when tested at 50 ng/mL.

Interference:

When Potential interferents (hemoglobin, triglycerides and bilirubin) were added at a minimum of ten folds of their physiological concentration to control sample, PAPP-A concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP7-P.

Interferents	Analyte Conc. (mg/mL)	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	9.3	415.328 781.587	410.137 802.814	-1.25 2.72
Triglygerides	33.8	415.328 781.587	441.731 783.105	6.357 0.194
Bilirubin	4.2	820.994 410.13	884.731 411.171	7.763 0.254

Method Comparison:

The PAPP-A ELISA has been compared to AnshLite™ PAPP-A chemiluminiscent assay (AL-206) using 45 pregnant female serum samples. Passing Bablok analysis of the results yielded the following Regression:
PAPP-A CLIA (AL-106) = 1.00 (AL-206) + 25.99
(r=0.985; P<0.0001)

REFERENCES

1. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
2. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
3. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

Research Use Only

Not for use *in-vitro* diagnostic procedures.

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