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 are reported at gestational ages between seven and thirteen weeks.

PRINCIPLE OF THE TEST

The PAPP-A ELISA is a quantitative one-step sandwich type immunoassay. In the first step Calibrators, Controls, unknown diluted samples, and horseradish peroxidase labelled antibody conjugate are added to anti-PAPP-A antibody coated microtiter wells and incubated. After the first 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-HRP conjugate 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 (Lyophilized)

Six vials, labeled A-F, containing concentrations of approximately 0-5000 ng/mL PAPP-A in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened vial at 2 to 8°C until the expiration date. Reconstitute calibrators A-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze at -20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

Note: *The PAPP-A concentration in the Ansh PAPP-A ELISA is traceable to the manufacturer's working calibrators.* ***The calibrator value has been corrected with a dilution factor of 150 to account for the sample dilution****.*

CTR-106-I and CTR-106-II PAPP-A Controls (Lyophilized)

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. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze at 20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

Conversion factor: *1 ng/mL of purified hetero-tetrameric PAPP-A (ht-PAPP-A) characterized by amino acid analysis in Ansh 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.*

PLT-101 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.

ECR-106 PAPP-A Antibody-Enzyme Conjugate Ready-To-Use (RTU)

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

SPD-106 Sample Diluent (10X)

One bottle, 12 mL, containing protein-based buffer with a nonionic detergent. Store at 2 to 30°C until expiration date. **Dilute 10-fold with deionized water prior to use.**

TMB-100 TMB Chromogen Solution

One bottle, 12 mL each, 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 each, 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 10–250 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. 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.

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Refer to package insert included with the product for exact specifications.

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. 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.[2]

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 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 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. **PAPP-A Calibrators A-F and PAPP-A Controls I & II**: Tap and reconstitute PAPP-A Calibrator A-F and PAPP-A Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
2. **Sample Diluent**: Sample Diluent should be diluted at a ratio of **1 part** into **9 parts** of the deionized water, according to the number of wells used. For one plate equivalent, pipet exactly **15mL** of the **SPD-106** into **135 mL** of the deionized water, mix well and label as sample diluent RTU.
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 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. **Sample Diluent Preparation**: Refer preparation of reagents, section 2.
2. **Manual assay:** For each unknown serum sample, label one 12 X 75 culture tube appropriately and add **1500 µL** of the sample diluent (SPD-106) to each tube. Add **10 µL** of the serum specimens to the pre-labeled tube and mix well.
3. Place the tubes in a tight-fitting rack and shake at a slow speed (450-550 rpm) at room temperature (23 ± 2°C) for **30 minutes**.
4. Vortex well. The samples are now ready to be assayed.

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. Reconstitute PAPP-A Calibrator A-F and PAPP-A Controls I & II each with 1 mL deionized water. Solubilize for **10 minutes**, Mix well.
2. Mark the microtitration strips to be used.
3. Pipet **50 µL** of the calibrators, controls, and unknown diluted samples (as described in the Sample Preparation section) to the appropriate wells.
4. Add **100 µL** of the antibody-enzyme conjugate ready-to-use to each well using a repeater pipette.
5. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **45 minutes** at room temperature (23 ± 2°C).
6. Aspirate and wash each well **5 times** with the wash solution **(350 µL/per well)** using an automatic microplate washer.
7. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid direct exposure to heat and sunlight.
8. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **10-12 min** at room temperature (23 ± 2°C).
   NOTE: Visually monitor the color development to optimize the incubation time.
9. Add **100 µL** of the stopping solution to each well using a repeater pipette.
10. 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.

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Refer to package insert included with the product for exact specifications.

RESULTS

NOTE: *The results in this package insert were calculated by plotting the* ***log optical density (OD) data on the y-axis and log PAPP-A concentration on X-axis*** *using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Optimum results can be obtained at incubation temperature of **23 ± 2°C**.
2. Calculate the mean OD for each calibrator, control, or diluted sample.
3. 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
4. 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.
5. Any sample reading higher than the highest calibrator should be appropriately diluted using sample diluent and re-assayed and corrected for the dilution factor.
6. 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 controls are printed on the calibration card.
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 Calibrators	Mean Absorbance	Conc (ng/mL)
A1, A2	A	0.008 (Blank)	0
B1, B2	B	0.029	18.0
C1, C2	C	0.15	90.0
D1, D2	D	0.55	344.0
E1, E2	E	1.82	1221.0
F1, F2	F	3.85	3300.0

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 ng/mL.

Conversion Factor

Conversion Factor: **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 18 replicates of calibrator A (0 ng/mL) and calibrator B (28.0 ng/mL) is 1.14 ng/mL.

Imprecision:

Reproducibility of the PAPP-A ELISA assay was determined in a study using three serum pools. The study included a total of 5 assays, three replicates of each of the serum pools per assay (n=15). Representative data are presented in the following table.

Sample	Mean Conc.	Total	
	(ng/mL)	%CV	SD
Pool-1	137.6	11.2	15.36
Pool-2	674.6	5.9	39.52
Pool-3	1709.9	4.8	81.74

Linearity:

Calibrator F and samples containing various PAPP-A levels were diluted in sample diluent RTU (SPD-106 RTU). 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	Average %Recovery
Calibrator F	Neat	3300.0			95%
	2	1650.0	1641.3	99%	
	4	825.0	741.8	90%	
	8	412.5	383.7	93%	
	16	206.3	197.0	95%	
	32	103.1	101.8	99%	
S1	Neat	2688.5			99%
	2	1344.2	1316.6	98%	
	4	672.1	634.7	94%	
	8	336.1	322.0	96%	
	16	168.0	166.8	99%	
	32	84.0	88.7	106%	
S2	Neat	1967.9			100%
	2	983.9	959.8	98%	
	4	492.0	466.4	95%	
	8	246.0	242.7	99%	
	16	123.0	127.7	104%	
	32	61.5	63.5	103%	

Analytical Specificity:

The antibody pair used in the assay detects Dimeric PAPP-A, Stanniocalcin-2, and PAPP-A Stanniocalcin-2. Other related molecules at the concentration in the table below did not show any significant cross-reaction.

Cross-reactant	Concentration (ng/mL)	%Cross-reactivity
Dimeric PAPP-A	50	49.71%
Pro-MBP	50	ND
PAPP-A2	50	ND
Alpha-2-Macroglobulin	50	ND
βhCG	50	ND
IhCG	50	ND
AFP	50	ND
Stanniocalcin-1	50	ND
Stanniocalcin-2	50	56.01%
PAPP-A Stanniocalcin-2	50	340.41%
LH	50	ND
FSH	50	ND

ND- Non-Detectable

Interference:

When potential interferents (hemoglobin, biotin, intralipids and bilirubin) were added at least at two times their physiological concentration to control sample, PAPP-A concentrations were within ±20% of the control as represented in the following table.

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Interferent	Interferent Dose	Sample PAPP-A (ng/mL)	Dosed Sample PAPP-A (ng/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	42.55	40.83	-4.04
	0.5 mg/mL	42.57	41.18	-3.27
Hemoglobin	1 mg/mL	1826.32	1852.97	1.46
	0.5 mg/mL	1883.98	1851.55	-1.72
Biotin	1200 ng/mL	38.37	36.96	-3.68
	600 ng/mL	40.48	40.83	0.87
Biotin	1200 ng/mL	1772.01	2061.40	16.33
	600 ng/mL	1948.76	1852.46	-4.94
Intralipids	20 mg/mL	49.14	46.39	-5.60
	10 mg/mL	46.04	40.48	-12.08
Intralipids	20 mg/mL	1692.46	1764.29	4.24
	10 mg/mL	1812.51	1879.71	3.71
Bilirubin	0.66 mg/mL	23.60	24.34	3.14
	0.2 mg/mL	35.19	35.55	1.01
Bilirubin	0.66 mg/mL	1242.21	1216.09	-2.10
	0.2 mg/mL	1726.56	1704.93	-1.25

Manufactured by:
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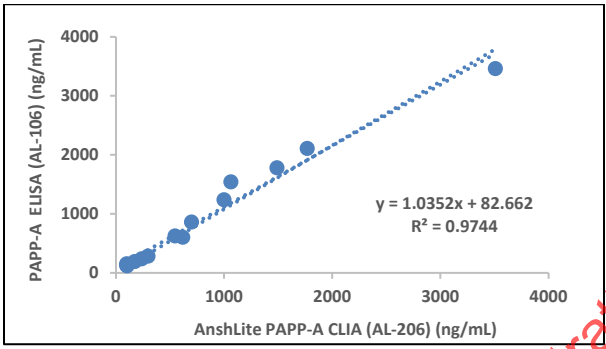
Method Comparison:

The PAPP-A ELISA has been compared to AnshLite™ PAPP-A chemiluminescent assay (AL-206) using 14 pregnant female serum samples.

PAPP-A ELISA (AL-106) = 82.662 + 1.0352 PAPP-A CLIA (AL-206)
(r=0.987)



Expected Value:

Serum samples were analyzed using Ansh Labs PAPP-A ELISA kit. The expected range for PAPP-A were calculated using 95% non-parametric estimation using Analyse-It® for Microsoft Excel as shown in table below.

Gestational Age	Gest. Age (wks., days)	No of specimens (n)	PAPP-A (ng/mL) 95% CI
1st Trimester	13,0 – 15,5	20	230.2 – 3823.6

Note: *It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.*

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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.

FOR RESEARCH USE ONLY

This assay is intended for in vitro diagnostic use. **Not for sale in U.S.A**

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