AnshLabs

picoPAPP-A ELISA

CE

AL-101-i

INTENDED USE

The picoPAPP-A enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of PAPP-A in human serum and other biological fluids. This assay is intended for *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

Pregnancy-associated plasma protein A (PAPP-A) is a large placenta-derived glycoprotein. During pregnancy it is produced in high concentrations by the trophoblast and released into maternal circulation. In addition to trophoblasts, PAPP-A expression has been reported in various tissues, including endometrium, testis, kidney, bone, colon, and other adult and fetal tissues[1,2,3]. 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[1,4,5,6,7]. Studies suggest that the PAPP-A form in non-pregnant females and males is dimeric and is not complexed with proMBP.

PRINCIPLE OF THE TEST

The picoPAPP-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 micro titer 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-101A - CAL-101F picoPAPP-A Calibrator A-F

Six vials, 0.5 mL each, labeled A-F containing concentrations of 0 - 10 ng/mL recombinant PAPP-A (rPAPP-A) in human serum 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.** The PAPP-A concentration in the picoPAPP-A calibrators is traceable to the manufacturer's working calibrators. 1 ng/mL of purified rPAPP-A characterized by amino acid analysis in picoPAPP-A assay yields 2.7 µIU/mL. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-101-I & CTR101-II picoPAPP-A Controls

Two vials, 0.5 mL each, labeled Levels I and II containing low and high concentrations of PAPP-A in human serum with a non-mercury preservative. .Refer to **calibration card** for exact concentrations. 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-101 picoPAPP-A Antibody-Enzyme Conjugate Concentrate

Dilute 10–30 minutes prior to use in PAPP-A conjugate diluent. 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.

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 450 nm, 405nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

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.

d) If external package is damaged, inspect the components inside for any other damage. Do not use if the components are damaged.

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[8].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[9] as a preservative. Pro-Clean 400 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.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the picoPAPP-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.

TEST PROCEDURE

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. **picoPAPP-A Antibody-Enzyme Conjugate Solution:** The picoPAPP-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.

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 **50 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.
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 1hr 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

***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 Controls and 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 picoPAPP-A Calibrator A and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. Multiply the value by a dilution factor.

LIMITATIONS

The reagents supplied in this kit are optimized to measure PAPP-A levels in human 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[11].

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- picoPAPP-A ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for picoPAPP-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	Mean Absorbance	Conc (ng/mL)
	Calibrators		
A1, A2	A	0.008 **(Blank)**	0
B1, B2	B	0.027	0.1
C1, C2	C	0.93	0.35
D1, D2	D	0.32	1.2
E1, E2	E	1.18	4
F1, F2	F	2.84	10

***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. To convert to µIU/mL: 1ng/mL of rPAPP-A = 2.7 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 0.037 ng/mL. The value was determined by processing seven serum samples in the range of 0.1 to 1.7 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 5% total imprecision is 0.1 ng/mL. The value was determined by processing eight samples in the range of 0.1-4.4 ng /mL with a minimum of twelve runs and three days in duplicates (n=24) following CLSI EP17 guidelines.

Imprecision:

Reproducibility of the picoPAPP-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	0.973	0.016	1.69%	0.002	0.22%	0.017	1.70%
Pool-2	1.553	0.028	1.82%	0.020	1.27%	0.034	2.21%
Pool-3	3.190	0.078	2.45%	0.012	0.39%	0.079	2.48%

Recovery

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

Sample	Endogenous Conc. (ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	0.603	1.05	1.01	96
		1.457	1.31	90
		1.829	1.591	87
2	0.707	1.15	1.134	99
		1.552	1.451	94
		1.919	1.766	92
3	0.73	1.171	1.122	96
		1.573	1.433	91
		1.939	1.748	90
3	0.836	1.272	1.228	97
		1.669	1.510	90
		2.031	1.771	87

Linearity

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

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
	Neat	4.154	N/A	N/A
	1:2	2.077	2.150	104
1	1:4	1.039	1.072	103
	1:8	0.519	0.570	110
	1:16	0.260	0.281	108
	Neat	3.925	N/A	N/A
	1:2	1.963	2.064	105
2	1:4	0.981	1.100	112
	1:8	0.491	0.550	112
	1:16	0.245	0.279	114
	Neat	3.784	N/A	N/A
	1:2	1.892	1.948	103
3	1:4	0.946	0.991	105
	1:8	0.473	0.520	110
	1:16	0.237	0.255	108
	Neat	4.079	N/A	N/A
	1:2	2.040	2.039	100
4	1:4	1.020	1.107	109
	1:8	0.510	0.556	109
	1:16	0.255	0.266	104

Analytical Specificity

The antibody pair used in the picoPAPP-A ELISA measures dimeric recombinant PAPP-A and PAPP-A/proMBP complex in equimolar concentration and does not cross react to PAPP-A2, MMP-9 and proMBP.

Interference:

When Potential interferents (hemoglobin, triglycerides and bilirubin) were added at twice 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	1.35	1.091 0.89	1.083 0.863	-0.733 -3.034
Triglygerides	5	1.091 0.89	1.052 0.863	-3.575 -3.034
Bilirubin	0.6	0.523 0.968	0.536 0.948	2.486 -2.066

Method Comparison:

The picoPAPP-A ELISA has been compared to picoPAPP-A CLIA assay (Method A) using 16 random male and female serum samples. Passing Bablok analysis of the results yielded the following Regression:

picoPAPP-A ELISA (AL-101) = 1.00 (picoPAPP-A CLIA) +0.07

(r=0.99; P<0.0001)

Expected Value:

Serum samples were analyzed using Ansh picoPAPP-A ELISA kit on site. The expected ranges for picoPAPP-A were calculated using 90-95% non-parametric estimation using Analyse-It® for Microsoft Excel.

Sample	Median Age	Median Conc. (ng/mL)	2.5 – 97.5th Percentile Conc (ng/mL)
Random Male and Female (N=667)	50	0.649	0.286 – 1.245

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

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FOR EXPORT ONLY

Symbols used with Ansh Labs Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	Caution	Vorsicht	mise en garde	precaución	attenzione
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Date of Manufacture	Herstellungsdatum	date de fabrication	fecha de manufactura	Data di produzione
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
	96-well plate	Platte mit 96 Vertiefungen	Plaque à 96 puits	placa de 96 pocillos	piastra a 96 pozzetti