PAPP-A Stanniocalcin-2 Complex ELISA

RUO

AL-166

INTENDED USE

The PAPP-A Stanniocalcin-2 Complex enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of PAPP-A Stanniocalcin-2 Complex in human 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

Mammalian stanniocalcin-2 (STC2) is a secreted polypeptide widely expressed in developing and adult tissues. However, although transgenic expression in mice is known to cause severe dwarfism, and targeted deletion of STC2 causes increased postnatal growth, its precise biological role has remained unknown. STC2 potently inhibits the proteolytic activity of the growth promoting metalloproteinase, pregnancy associated plasma protein-A (PAPP-A). Proteolytic inhibition requires covalent binding of STC2 to PAPP-A, and is mediated by a disulfide bond, which involves Cys120 of STC2. Binding of STC2 prevents PAPP-A cleavage of insulin-like growth factor binding protein (IGFBP)-4 and hence release within tissues of bioactive IGF, required for normal growth. Concordantly, STC2 efficiently inhibits PAPP-A-mediated IGF receptor signaling in vitro, and that transgenic mice expressing a mutated variant of STC2, STC2(C120A), which is unable to inhibit PAPPA, grow like wild type mice. STC2 is a novel proteinase inhibitor and a previously unrecognized extracellular component of the IGF system.[1]

PRINCIPLE OF THE TEST

The PAPP-A Stanniocalcin-2 complex ELISA is a quantitative, three-step sandwich type immunoassay. In the first step, Calibrators, Controls, and unknown samples are added to STC2 antibody coated microtiter 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 STC2 complex in the samples and calibrators.

MATERIALS SUPPLIED

CAL-166 A **PAPP-A STC2 Complex Calibrator A/Sample Diluent**

One bottle, 6 mL, labeled PAPP-A STC2 complex Calibrator A/Sample Diluent, containing 0 ng/mL PAPP-A STC2 complex in protein-based buffer with non-mercury preservative. Store at 2 to 8°C until the expiration date.

CAL-166 B-F **PAPP-A STC2 Complex Calibrator B-F**

Four vials, labeled PAPP-A STC2 complex calibrator B-F containing concentrations of 0.77 – 50 ng/mL PAPP-A STC2 complex in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Reconstitute calibrators B-F 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. 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-166-I & CTR-166-II **PAPP-A STC2 Complex Controls (Lyophilized)**

Two vials, labeled Levels I and II containing low and high concentrations of PAPP-A STC2 Complex in protein-based buffer with a non-mercury preservative. Refer to **calibration card** for exact concentrations. 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-143 **Stanniocalcin-2 Antibody Coated Microtitration Strips**

One strip holder, containing 96 polystyrene microtitration wells with Stanniocalcin-2 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-166 **PAPP-A STC2 Complex Biotin Conjugate Concentrate**

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

SAR-166 **PAPP-A STC2 Complex 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 2-8°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 630nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Repeater 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.[2]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[3] 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.

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.[4]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the PAPP-A Stanniocalcin-2 complex 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

Preparation of Reagents

1. **PAPP-A STC2 Complex Calibrators B-F and PAPP-A STC2 Complex Controls I & II:** Tap and reconstitute PAPP-A STC2 Complex Calibrator B-F and PAPP-A STC2 Complex Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution.
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. **PAPP-A STC2 Complex Biotin Conjugate Solution:** The PAPP-A STC2 Complex 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 pregnancy samples should be diluted 1:5 in the ng/mL (Calibrator A)*
- *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 Calibrators A-F, 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 **2 hrs** at room temperature (23±2°C).
5. During the last 10-30 minutes of incubation, prepare the Biotin Conjugate Solution by diluting the PAPP-A STC2 complex 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 (prepared as per step 5) to each well using a precision pipette.
8. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **1 hr** 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 STC2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the PAPP-A STC2 complex concentrations of the controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding PAPP-A STC2 complex concentrations.
4. Any sample reading higher than the highest calibrator should be appropriately diluted using PAPP-A STC2 complex 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, if applicable.

LIMITATIONS

The reagents supplied in this kit are optimized to measure PAPP-A STC2 complex 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.[5]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- PAPP-A STC2 Complex ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for PAPP-A STC2 Complex 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	0.029 **(Blank)**	0
B1, B2	B	0.072	0.77
C1, C2	C	0.17	1.83
D1, D2	D	0.6	9.58
E1, E2	E	1.51	23.37
F1, F2	F	2.68	50

***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 in the assay as calculated by the interpolation of mean plus two standard deviation of 18 replicates of calibrator A (0 ng/mL) and low calibrator (0.77 ng/mL) is 0.014 ng/mL.

Imprecision:

Reproducibility of the PAPP-A STC2 Complex ELISA assay was determined in a study using two kit controls and two serum pools assayed in 54 replicates. Representative data were calculated and are presented in the following table.

Sample	Mean conc.	Within run		Between run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Control-I	4.39	0.13	2.96%	0.13	2.96%	0.18	4.18%
Control-II	14.00	0.36	2.56%	0.24	1.75%	0.43	3.10%
Pool-1	2.29	0.05	2.17%	0.05	2.22%	0.07	3.10%
Pool-2	6.24	0.23	3.69%	0.49	7.85%	0.54	8.68%

Recovery

Recombinant PAPP-A STC2 (Calibrator E) was spiked into one male and one female serum samples at three different levels and assayed. The spike recovery is shown below.

Sample ID	Endogenous Conc.(ng/mL)	Expected Conc. ng/mL	Observed Conc. (ng/mL)	%Recovery
Male	0.446	1.421	1.215	85%
		2.317	1.966	85%
		3.143	2.814	90%
Female	0.859	1.817	1.640	90%
		2.697	2.404	89%
		3.507	3.092	88%

Linearity

Multiple dilutions of the two serum samples containing various PAPP-A STC2 Complex levels were diluted with Calibrator A. The percentage recovery on individual samples is represented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Value in	Observed Value in	% Recovery
First Trimester	NEAT	6.069	NA	NA
	2	3.035	3.140	103%
	4	1.517	1.664	110%
	8	0.759	0.865	114%
	16	0.379	0.432	114%
	32	0.190	0.216	114%
Second Trimester	NEAT	20.863	NA	NA
	2	10.432	11.099	106%
	4	5.216	5.743	110%
	8	2.608	2.835	109%
	16	1.304	1.411	108%
	32	0.652	0.698	107%
PAPP-A STC2 Complex Antigen	NEAT	23.37	NA	NA
	2	11.685	11.328	97%
	4	5.843	5.279	90%
	8	2.921	2.547	87%
	16	1.461	1.241	85%
	32	0.730	0.631	86%

Analytical Specificity:

The antibody pair used in the assay is specific to PAPP-A STC2 complex. Other related molecules at the concentration in the table below did not show any significant cross-reaction.

Cross-Reactant	Concentration	% Cross-reactivity
Stanniocalcin-1	300 ng/mL	ND
Stanniocalcin-2	50 ng/mL	ND
Dimeric PAPP-A	500 ng/mL	ND
Alpha-2-Macroglobulin	150 ng/mL	ND
proMBP	100 ng/mL	ND

REFERENCES

1. Malene R. Jepsen, Søren Kløverpris , Jakob H. Mikkelsen , Josefine H. Pedersen, Ernst-Martin Füchtbauer, Lisbeth S. Laursen, and Claus Oxvig JBC Papers in Press. Published on December 22, 2014 as Manuscript M114.611665.
2. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
3. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http:// www.cdc.gov/niosh.
4. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
5. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

12. FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

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