AnshLabs

Stanniocalcin-2 ELISA

RUO

AL-143

INTENDED USE

The Stanniocalcin-2 (STC2) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Stanniocalcin-2 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

Stanniocalcins, STC1 and STC2 are glycoprotein hormones. They were originally identified in fish, where they regulate calcium and phosphate homeostasis. Human STC2 is a 33.3 kDa protein, known to form disulfide-linked homodimers. The amino acid sequence of STC2 is only 30% identical to STC1, but the glycosylation sites and cysteine residues are conserved. STC2 is phosphorylated by Casein kinase 2. STC2 is widely expressed with most abundant protein expression in brain, adrenal gland, kidney, prostate and small intestine. [1]

STC2 function in hormone signaling is indicated by reports showing inhibition of ovarian progesterone biosynthesis and transactivation of androgen receptor.[2,3] STC2 exhibits anti-apoptotic functions in cells subjected to endoplasmic reticulum and hypoxic stress by a mechanism involving inhibition of plasma membrane store-operated calcium entry.[4,5] STC2 overexpression mice exhibit growth restriction, while knockout mice are larger than wild-type littermates.[1] STC2 function in growth regulation was demonstrated by its ability to interact with PAPP-A, potentially inhibiting its proteolytic activity towards IGFBP-4 and causing reduced IGF signaling. [6,7,8] STC2-mediated PAPP-A inhibition was also reported to reduce atherosclerosis in hypercholesterolemic mice. [9]

In breast cancer STC2 is induced by estrogen and STC2 expression levels correlate with levels of estrogen receptor. STC2 function in different cancers may involve regulation of cell proliferation, neoplastic transformation and endothelial invasion. STC2 also plays a role in epithelial-mesenchymal transition by activation of MAPK/ERK signaling in hypoxic ovarian cancer cells. STC2 is upregulated in several cancers including gastric cancer, lung cancer, renal cell carcinoma, colorectal cancer, castration-resistant prostate cancer and cervical cancer.[1] Serum STC2 levels may serve as a valuable research tool for diagnosis and prognosis of several cancers and growth defects.

PRINCIPLE OF THE TEST

The Stanniocalcin-2 ELISA is a quantitative two-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Stanniocalcin-2 antibody coated microtiter wells and incubated. After the 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 Stanniocalcin-2 in the samples and calibrators.

MATERIALS SUPPLIED

CAL-143A Stanniocalcin-2 Calibrator A/Sample Diluent

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

CAL-143B - CAL-143F Stanniocalcin-2 Calibrator B-F (Lyophilized)

Five vials, labeled Stanniocalcin-2 calibrator B-F containing concentrations of 1 - 50 ng/mL Stanniocalcin-2 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-143-I & CTR143-II Stanniocalcin-2 Controls (Lyophilized)

Two vials, labeled Levels I and II containing low and high concentrations of stanniocalcin-2 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 stripholder, 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.

ECC-143 Stanniocalcin-2 Antibody-Enzyme Conjugate Concentrate

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

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. [10]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[11] 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.

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. [12]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Stanniocalcin-2 ELISA. It is the responsibility of the user 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. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
2. **Stanniocalcin-2 Antibody-Enzyme Conjugate Solution:** The Stanniocalcin-2 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 10–30 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 (23 ± 2°C). 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 repeater 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. 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 (350 µL per well)** with the wash solution using an automatic microplate washer.
7. Add **100 µL** of the **Antibody-enzyme conjugate solution** to each well using a repeater 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 (350 µL per well)** with the wash solution using an automatic microplate washer.
10. Add **100 µL** of the **TMB chromogen solution** to each well using a repeater pipette. *Avoid direct exposure to heat and sunlight.*
11. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    ***NOTE**: Visually monitor the color development to optimize the incubation time.*
12. Add **100 µL** of the **Stopping solution** to each well using a repeater 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 **log optical density (OD) data on the y-axis and log Stanniocalcin-2 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 Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Stanniocalcin-2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the Stanniocalcin-2 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Stanniocalcin-2 concentrations.
5. Any sample reading higher than the highest calibrator should be appropriately diluted using Stanniocalcin-2 Calibrator A and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if applicable.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Stanniocalcin-2 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. [13]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Stanniocalcin-2 ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Stanniocalcin-2 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	0.01 **(Blank)**	0
B1, B2	B	0.086	0.85
C1, C2	C	0.21	2.12
D1, D2	D	0.89	9.5
E1, E2	E	2.12	25
F1, F2	F	3.65	55

***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 44 replicates of calibrator A (0 ng/mL) and low calibrator (0.85 ng/mL) is 0.033 ng/mL.

Imprecision:

Reproducibility of the Stanniocalcin-2 ELISA assay was determined in a study using two kit controls and two serum pools assayed in 54 replicates. 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
Control-I	4.42	0.46	10.43%	0.10	2.35%	0.47	10.69%
Control-II	14.70	0.77	5.23%	0.94	6.38%	1.21	8.25%
Pool-1	15.44	0.72	4.63%	0.90	5.85%	1.15	7.46%
Pool-2	22.51	0.90	3.99%	1.43	6.37%	1.69	7.52%

Recovery

Calibrator F was spiked into 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	29.599	33.41	32.13	96%
		32.14	31.43	98%
		30.87	30.18	98%
Female	23.062	27.85	25.84	93%
		26.26	25.09	96%
		24.66	22.02	89%
First Trimester	17.263	22.92	20.42	89%
		21.04	18.77	89%
		19.15	18.49	97%
Second Trimester	18.016	23.56	23.17	98%
		21.71	21.46	99%
		19.87	20.18	102%

Linearity

Based on NCCLS EP-6-P, multiple dilutions of the four serum samples containing various Stanniocalcin-2 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
Male	NEAT	29.60	NA	NA
	2	14.80	15.51	105%
	4	7.40	7.92	107%
	8	3.70	3.79	102%
	16	1.85	1.93	104%
Female	NEAT	23.06	NA	NA
	2	11.53	11.27	98%
	4	5.77	5.74	100%
	8	2.88	2.79	97%
	16	1.44	1.36	94%
First Trimester	NEAT	17.26	NA	NA
	2	8.63	9.12	106%
	4	4.32	4.47	104%
	8	2.16	2.28	105%
	16	1.08	1.17	108%
Second Trimester	NEAT	13.33	NA	NA
	2	6.67	6.71	101%
	4	3.33	3.47	104%
	8	1.67	1.73	104%
	16	0.83	0.85	102%

Interference:

When Potential interferents (hemoglobin, triglycerides and bilirubin) were added at twice their physiological concentration to control sample, Stanniocalcin-2 concentration were within ± 13% 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	26.03	26.75	2.78
		33.91	33.34	-1.69
		23.46	24.37	3.88
		32.23	34.10	5.79
		19.87	21.40	7.69
		10.52	11.15	5.98
Triglycerides	5	26.03	27.74	6.58
		33.91	32.91	-2.96
		23.46	23.63	0.70
		32.23	36.27	12.52
		19.87	22.41	12.79
		10.52	11.46	8.88
Bilirubin	0.6	26.76	29.23	9.24
		32.18	34.21	6.31
		21.32	23.65	10.90
		31.63	32.45	2.61
		20.20	20.56	1.77
		10.61	11.30	6.52

Analytical Specificity:

The antibody pair used in the assay is specific to Stanniocalcin-2. Other related molecules at the concentration in the table below did not show any significant cross-reaction.

Cross-Reactant	Concentration	% Cross-reactivity
Stanniocalcin-1	50 ng/mL	ND
Alpha-2-Macroglobulin	500 ng/mL	ND
Dimeric PAPP-A	58 ng/mL	ND
Heterotetrameric PAPP-A	36 ng/mL	ND
proMBP	500 ng/mL	ND

Expected Value:

Samples were analyzed using Stanniocalcin-2 ELISA. The circulating levels of Stanniocalcin-2 in males and females are represented in the following table.

Samples	Number of specimens	Median Age	STC2 Median (ng/mL)	STC2 Range (ng/mL)
Female (Age: 14-91 years)	20	43	20.80	3.61 - 43.29
Male (Age: 18-89 years)	20	51	24.21	9.69 - 51.18
First Trimester (Gestational age: 11-14 weeks)	19	13	16.18	11.05 - 30.92
Second Trimester (Gestational age: 15-20 weeks)	20	17	13.52	6.86 - 17.94

The expected ranges for Stanniocalcin-2 in pediatric male samples in the age range of 3.0 – 18.0 years were calculated using 95% non-parametric estimation. A total of 404 samples in Pubic Hair Tanner stages 1 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Pubic Hair Tanner Stage	No. of specimens (n)	Median Conc. (ng/mL)	Stanniocalcin-2 (ng/mL) 95% CI
1	218	21.5	14.0 - 35.5
2	54	23.6	14.7 - 31.1
3	32	25.0	15.1 - 47.0
4	50	24.2	16.7 - 37.2
5	50	26.1	17.6 - 41.7

The expected ranges for Stanniocalcin-2 in pediatric female samples in the age range of 2.4 – 18.0 years were calculated using 95% non-parametric estimation. A total of 432 samples in Breast Tanner stages 0 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Breast Tanner Stage	No. of specimens (n)	Median Conc. (ng/mL)	Stanniocalcin-2 (ng/mL) 95% CI
0	15	19.5	16.9 - 25.6
1	174	21.2	15.3 - 34.9
2	61	23.2	15.5 - 29.8
3	58	27.4	19.1 - 43.7
4	53	26.2	18.2 - 37.2
5	71	27.6	5.0 - 44.1

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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12. FOR RESEARCH USE ONLY

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Ansh Labs.
445 Medical Center Blvd.
Webster, TX 77598-4217 U.S.A.