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ab222880 Human Stanniocalcin 2 / STC-2 ELISA Kit

For the quantitative measurement of human Stanniocalcin 2 / STC-2 in plasma and serum samples.

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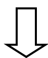
1. Overview

The Human Stanniocalcin 2 / STC-2 ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab222880) is designed for detection of Stanniocalcin 2 / STC-2 in human plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Stanniocalcin 2 / STC-2 in approximately 4 hours. A polyclonal antibody specific for human Stanniocalcin 2 / STC-2 has been pre-coated onto a 96-well microplate with removable strips. Stanniocalcin 2 / STC-2 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human Stanniocalcin 2 / STC-2, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

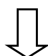
Stanniocalcin 2 / STC-2, also known as stanniocalcin-related protein, is a secreted homodimeric glycoprotein that belongs to the stanniocalcin family. Stanniocalcin 2 / STC-2 contains 302 amino acid residues with 34% STC-1 identity and a molecular mass of 32 kDa. The N terminus of Stanniocalcin 2 / STC-2 contains a signal peptide followed by a cysteine-rich region that may mediate homodimerization. In the kidney, the function of Stanniocalcin 2 / STC-2 on phosphate transport appears to be the opposite to that of STC-1. Stanniocalcin 2 / STC-2 is phosphorylated by casein kinase-2 on serine residues and can inhibit phosphorylation of cell-surface proteins. It is expressed in a wide variety of tissues, including the pancreas, skeletal muscle, and small intestine. Stanniocalcin 2 / STC-2 may play a role in the regulation of renal and intestinal calcium and phosphate transport, cell metabolism, and cellular calcium/phosphate homeostasis.

2. Protocol Summary

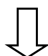
Prepare all reagents, samples, and standards as instructed



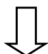
Add 50 µL standard or sample to appropriate wells and incubate for 2 hours



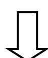
Wash wells, add 50 µL Biotinylated Antibody to each well and incubate for 2 hour



Wash wells, add 50 µL Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes



Wash wells, add 50 µL Chromogen Substrate to each well and incubate for 20 minutes



Add 50 µL Stop Solution to each well and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from Biotinylated Human Stanniocalcin 2 / STC-2, Human Stanniocalcin 2 / STC-2

Standard and 100X Streptavidin-Peroxidase Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Stanniocalcin 2 / STC-2 coated Microplate (12 x 8 wells)	96 wells	+4°C
Human Stanniocalcin 2 / STC-2 Standard	1 Vial	-20°C
Biotinylated Human Stanniocalcin 2 / STC-2 antibody	180 µL	-20°C
10X Diluent N Concentrate	30 mL	+4°C
1X Standard Diluent	2 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	8 mL	+4°C
Stop Solution	12 mL	+4°C
Sealing Tapes	3 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel).
- Deionized or distilled reagent grade water.

8. Technical Hints

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 **10X Diluent N Concentrate:**
When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Prepare 1X Diluent N by diluting Diluent N Concentrate 1 in 10 with reagent grade water to produce a 1X solution. Store for up to 30 days at 2-8°C.

9.2 **Biotinylated Human Stanniocalcin-2:**
Spin down the antibody briefly and dilute the desired amount of the antibody 1 in 30 with 1X Diluent N to produce a 1X solution. The undiluted antibody should be stored at -20°C.

9.3 **20X Wash Buffer Concentrate:**
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Wash Buffer by diluting Wash Buffer Concentrate 1 in 20 with reagent grade water.

9.4 **100X Streptavidin-Peroxidase Conjugate:**

Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 1 in 100 with 1X Diluent N. The undiluted conjugate should be stored at -20°C.

9.5 **Standard Diluent (2 mL):**

Ready to use. Store at +4°C.

9.6 **Chromogen Substrate (8 mL):**

Ready to use. Store at +4°C.

9.7 **Sealing Tapes (3 units):**

Ready to use. Store at +4°C.

9.8 **Stop Solution (12 mL):**

Ready to use. Store at +4°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the Stanniocalcin 2 / STC-2 to generate a 20 ng/mL **Stock**.

10.1.1 First consult the Stanniocalcin 2 / STC-2 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Standard Diluent to add when resuspending the Stanniocalcin 2 / STC-2 Standard vial to produce a 20 ng/mL Stanniocalcin 2 / STC-2 **Stock** by using the following equation:

C_S = Starting mass of Stanniocalcin 2 / STC-2 Standard stock (see vial label) (ng)

C_F = 20 ng/mL Stanniocalcin 2 / STC-2 Standard Stock final required concentration

V_D = Required volume of 1X Standard Diluent for reconstitution (µL)

Calculate total required volume 1X Standard Diluent for resuspension:

$$(C_S / C_S) * 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 10 ng of Stanniocalcin 2 / STC-2 Standard in vial

C_F = 20 ng/mL Stanniocalcin 2 / STC-2 stock final concentration

V_D = Required volume of 1X Standard Diluent for reconstitution

(10 ng / 20 ng/mL) * 1,000 = 500 µL

10.1.3 First briefly centrifuge the Stanniocalcin 2 / STC-2 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Stanniocalcin 2 / STC-2 Standard vial by adding the appropriate calculated amount VD of 1X Standard Diluent to the vial to generate the 10 ng/mL Stanniocalcin 2 / STC-2 **Stock.** Mix gently and thoroughly.

10.2 Allow the reconstituted 20 ng/mL Stanniocalcin 2 / STC-2 **Stock** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Dilute the **Stock** 2-fold with Diluent N to produce a 10 ng/mL working solution (**Standard #1**).

10.4 Label seven tubes #2 – 8.

10.5 Prepare duplicate or triplicate standard points by serially diluting from the 10 ng/mL **Standard #1** with 1X Diluent N to produce 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/mL).

10.6 Add 120 µL of 1X Diluent N to tube #2 – 8.

10.7 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.8 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.9 Using the table below as a guide, prepare subsequent serial dilutions.

Standard #	**Volume to dilute (µL)**	**Volume Diluent N (µL)**	**STC-2 (ng/mL)**
1	120 µL working solution from Step 10.3		10
2	120 µL Standard #1	120	5
3	120 µL Standard #2	120	2.5
4	120 µL Standard #3	120	1.25
5	120 µL Standard #4	120	0.625
6	120 µL Standard #5	120	0.313
7	120 µL Standard #6	120	0.156
8 (Blank)	N/A	120	0

11. Sample Preparation

11.1 **Plasma:**

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 4-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 **Serum:**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 4-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and store in a vacuum desiccator.

12.3 Add 50 µL of all sample or standard to appropriate wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µl of Wash Buffer manually. Wash by aspirating or decanting from wells then dispensing 200 µL Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid. If using a machine, wash six times with 300 µl of Wash Buffer.

12.5 Add 50 µl of 1X Biotinylated human Stanniocalcin 2 / STC-2 antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 2 hours.

12.6 Wash the microplate as described above (12.4).

12.7 Add 50 µl of 1X Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above (12.4).

12.10 Add 50 µl of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have

formed and incubate in ambient light for 30 minutes or till the optimal blue color density develops.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

Δ **Note:** that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13. Calculations

13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

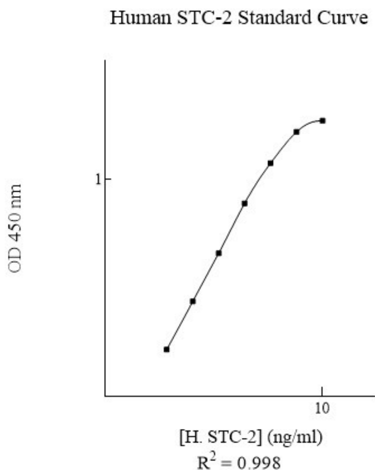


Figure 1. Example of human Stanniocalcin 2 / STC-2 standard curve in 1X Diluent N. The Stanniocalcin 2 / STC-2 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

15. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 70 pg/ml. The MDD was determined by calculating the mean of zero standard and adding 2 standard deviations then extrapolating the corresponding concentration.

PRECISION –

Intra-assay precision was determined by testing three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
Average CV (%)	4.8%	10.7%

RECOVERY –

Standard Added Value	0.313-5 ng/ml
Recovery (%)	89 – 111 %
Average Recovery (%)	97%

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Serum	Plasma
2	94%	92%
4	99%	101%
8	107%	105%

16. Assay Specificity

This kit recognizes human Stanniocalcin 2 / STC-2 protein in plasma, serum, and cell culture samples.

CROSS REACTIVITY

Species	Cross Reactivity (%)
Dog	None
Bovine	None
Equine	30%
Monkey	70%
Mouse	20%
Rat	30%
Swine	30%
Rabbit	None

17. Species Reactivity

This kit recognizes human, monkey, mouse, rat and pig Stanniocalcin 2 / STC-2 protein.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.

	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

19. Notes

Technical Support

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