Product Manual

Human Calcitonin ELISA Kit

Catalog Numbers

MET- 5062	96 assays
MET- 5062- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Calcitonin is a polypeptide hormone involved in calcium and phosphorus metabolism. The 32 amino acid protein is produced in the neuroendocrine C-cells, or parafollicular cells, of the thyroid. Non-mammals such as birds or fish produce the hormone in cells of the ultimobranchial body. Calcitonin lowers blood calcium levels by inhibiting osteoclast activity in the bones and inhibiting renal tubular reabsorption of calcium and phosphate and allowing them to excrete through the urine. The hormone will prevent calcium absorption from the intestine and increase calcium bone deposition. The hormone's activity counteracts the actions of parathyroid hormone (PTH), which acts to raise blood calcium levels (Figure 1). Its actions protect against calcium loss during periods of calcium mobilization, such as pregnancy and lactation. It may also be involved in the central nervous system regulation of feeding and appetite.

Calcitonin's importance has not been fully elucidated. While few symptoms occur with high or low calcitonin levels, high levels have been associated with medullary thyroid cancer of the parafollicular cells. Calcitonin is used as a treatment in bone calcium loss diseases such as osteoporosis and in Paget's disease, where repair and recycling of bone has been disrupted.

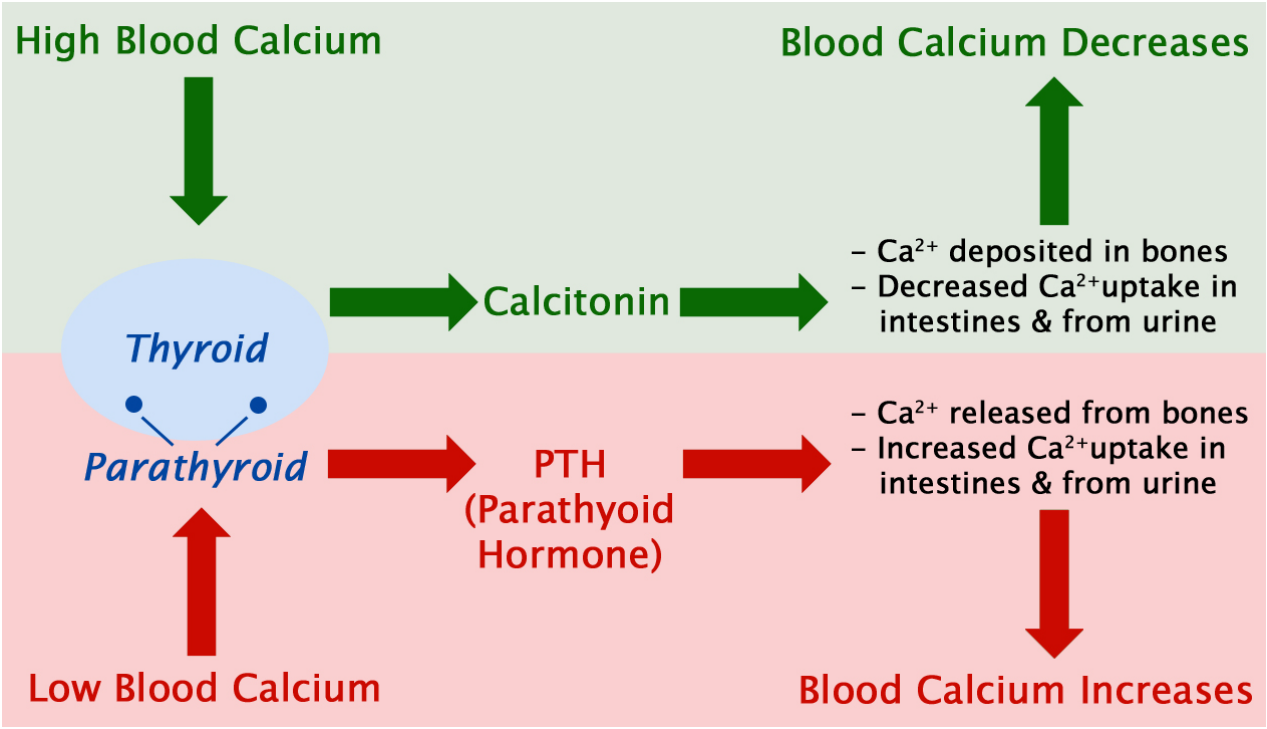

Figure 1: Blood Calcium Regulation by Calcitonin (top) and PTH (bottom).

Cell Biolabs' Human Calcitonin ELISA Kit is an enzyme immunoassay developed for detection and quantitation of human calcitonin. The kit utilizes a recombinant human calcitonin standard and has a detection sensitivity limit of ~2 pg/mL. Each kit provides sufficient reagents to perform up to 96 assays including the standard curve and samples.

Assay Principle

This assay is based on a sandwich ELISA format. Calcitonin present in samples or standards binds to the anti-calcitonin antibodies pre-adsorbed on the microtiter plate. Next, a biotinylated anti-calcitonin antibody is added to the plate well and binds to the captured calcitonin. A streptavidin-enzyme conjugate is then added, which binds to the biotin of the second antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of calcitonin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified recombinant human calcitonin. Sample concentration is then determined by comparing to the known values of the standard curve.

Related Products

1. CBA-290: CytoSelect™ 96-Well Adipogenesis Assay Kit
2. MET-5030: NAD^+ / NADH Assay Kit (Fluorometric)
3. MET-5031: $NADP^+$ / NADPH Assay Kit (Fluorometric)
4. MET-5051: Human Thyroid-Stimulating Hormone (TSH) ELISA Kit
5. MET-5052: Human Adiponectin ELISA Kit
6. MET-5057: Human Leptin ELISA Kit
7. MET-5063: Human Insulin ELISA Kit
8. STA-384: Total Cholesterol Assay Kit (Colorimetric)
9. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
10. STA-680: Glucose Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Calcitonin Antibody Coated Plate (Part No. 50621B): One strip well 96-well plate
2. Anti-Calcitonin Biotinylated Antibody (1000X) (Part No. 50622D): One 10 µL vial of anti-calcitonin antibody
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL tube
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

1. Calcitonin Standard (Part No. 50623D): One 10 µL vial of 5 µg/mL human calcitonin

Materials Not Supplied

1. Calcitonin samples: human serum, plasma, lysates
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store Calcitonin Standard at -80ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Calcitonin Biotinylated Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Anti-Calcitonin Biotinylated Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples can be diluted if necessary with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples can be diluted if necessary with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

Preparation of Standard Curve

1. Prepare fresh standards by diluting the Calcitonin Standard stock tube from 5 µg/mL to 5 ng/mL (1:1000) in Assay Diluent (Example: Add 2 µL of Calcitonin Standard stock tube to 1.998 mL of Assay Diluent).
2. Prepare a series of the remaining calcitonin standards in the concentration range of 125 pg/mL – 2 pg/mL by diluting the 5 ng/mL according to Table 1 below.

Standard Tubes	5 ng/mL Human Calcitonin Standard (µL)	Assay Diluent (µL)	Calcitonin (pg/mL)
1	25	975	125
2	500 of Tube #1	500	62.5
3	500 of Tube #2	500	31.3
4	500 of Tube #3	500	15.6
5	500 of Tube #4	500	7.8
6	500 of Tube #5	500	3.9
7	500 of Tube #6	500	2.0
8	0	500	0

Table 1. Preparation of Calcitonin Standard Curve.

Note: Do not store diluted calcitonin standard solutions.

Assay Protocol

Note: Each calcitonin standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of calcitonin standards or samples to the Anti-Calcitonin Antibody Coated Plate. Each sample, standard, blank, and control should be assayed in duplicate.
2. Incubate 1 hour at room temperature on an orbital shaker.
3. Remove the solution from the wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Anti-Calcitonin Biotinylated Antibody to each well.
5. Incubate 1 hour at room temperature on an orbital shaker.
6. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.

8. Incubate 1 hour at room temperature on an orbital shaker.
9. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
10. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Human Calcitonin ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

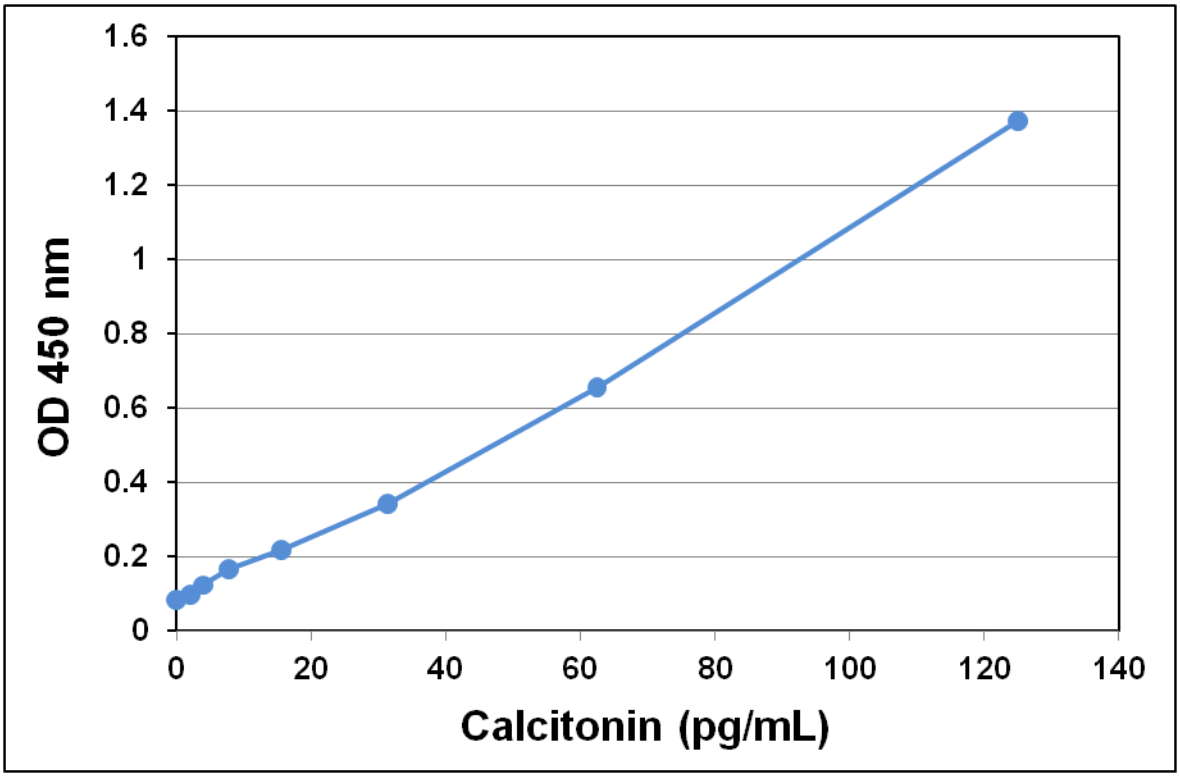

Figure 2: Human Calcitonin ELISA Standard Curve

References

1. Austin, L.A., et al. (1981) *N. Engl. J. Med.* **304**: 269-278.
2. Brain, S.D., et al. (1985) *Nature* **313**: 54-56.
3. Foster, G.V., et al. (1964) *Nature* **202**: 1303-1305.
4. Lee, S.M., et al. (2016) *J. Biol. Chem.* **291(16)**: 8686-700.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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