# Human Calcitonin ELISA Kit

Enzyme Immunoassay for the quantification of Calcitonin in human serum

Catalog number: ARG80776

For research use only. Not for use in diagnostic procedures.

# TABLE OF CONTENTS



**MANUFACTURED BY:**

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

# INTRODUCTION

## Biological activities

Calcitonin (CT) is a 32 amino acid peptide hormone secreted by the para-follicular C-cells of the thyroid gland under serum calcium control. After acute administration, this peptide acts as a potent hypocalcemic and hypophosphatemic hormone by increasing renal calcium clearance and reducing bone resorption. However, its precise physiological role in bone metabolism is not yet fully understood. Various forms of CT may be detected in blood samples, including a CT monomer, an oxidized monomer, a dimer, higher molecular weight forms, and possibly precursor of CT. The concentrations of these peptides vary with clinical status, renal function and tissular origin of CT (normal or ectopic production). Medullar thyroid carcinoma (MTC) is a malignant tumor, developed from the C-cells, secreting calcitonin in large excess. This disease occurs either as a sporadic (80%) or a familial (20%) form, which is transmitted as an autosomal dominant gene or as a component of multiple endocrine neoplasia (IIb). Moderate hypercalcitoninemia is also observed in pregnancy, pernicious anaemia, renal failure and during the neonatal period. Preferably, monomer form of CT is detected in this assay.

## Clinical Application

The measurement of CT is used for:

- Diagnosis of medullary thyroid carcinoma (MTC).
- Follow up of malignant tumors, to check the success of surgery and to

monitor for recurrence.

- Diagnosis of the preclinical cases of the familial forms of MTC (MEN II or Sipple syndrome) by the use of stimulation tests (calcium or pentagastrin).
- Study of the pathophysiology of the calcium-phosphate and bone metabolism.

## PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. A specific Calcitonin monoclonal antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells together with HRP-labeled Calcitonin antibody where a sandwich will be formed between Calcitonin present in the samples and the monoclonal antibodies. After washing away any unbound substances, a substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of Calcitonin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Calcitonin in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 12 X 8 strips | 4°C |
| Standard 0-5 | 6 vials (Lyophilized) Add 0.5 ml distilled water to reconstitute | 4°C |
| Control 1-2 | 2 vials (Lyophilized) Add 0.5 ml distilled water to reconstitute | 4°C |
| Conjugation Buffer | 6 ml (Ready-to-use) | 4°C |
| 50X HRP-Antibody Conjugate | 1 vial (0.125ml) | 4°C |
| CT-free Human Serum | 1 vial (Lyophilized) Add buffer B to reconstitute | 4°C |
| Buffer B | 8 ml (Ready-to-use) | 4°C |
| 200X Wash buffer | 10 ml | 4°C |
| TMB substrate | 12 ml (Ready-to-use) | 4°C (Protect from light) |
| STOP solution | 12 ml (Ready-to-use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.

- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 200X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Haemolysed and lipemic samples should be avoided.

## REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- Standard 0-5: Reconstitute Standard 0-5 with 0.5ml distilled water. Concentration after reconstitution is written on the vial label.
- Controls: Reconstitute Controls with 0.5ml distilled water.
- Working HRP conjugate solution: Prepare an adequate volume of conjugate solution by adding for example 40µl of 50X concentrated HRP

conjugate to 2ml conjugate buffer. Use vortex to homogenize. Fresh preparation is recommended.

- **CT Free Serum:** Reconstitute CT Free Human Serum with Buffer B as indicated on the vial label. Allow to dissolve completely. Mix gently by inversion.
- **Samples:** Samples can be diluted with CT Free Serum if necessary. Do not use haemolyzed or lipemic samples with this kit.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls, standards and samples in duplicate into wells.
3. Add 50 µl working HRP-conjugate solution into all wells.
4. Cover wells and incubate for 18 hours at 2-8°C.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark (TMB Reagent should be added within 15 minutes following the washing steps).

7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450 nm immediately,

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

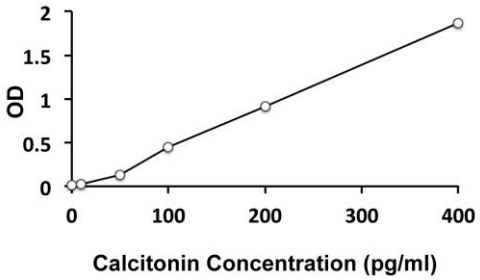
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The mean MDD was 0.7 pg/ml.

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.8% and inter-assay precision was 5.45%.

### Specificity

No cross-reactivity has been found with the following factors:

CGRP, Salmon-calcitonin, PDN21, Procalcitonin N terminal.

### Recovery

99-105%