FAQ: Frequently Asked Questions

Device: Calcitonin ELISA (REF 7024/7024BU)

Sample Type

What type of samples can I collect and test?
The determination of Calcitonin should be performed with serum. Collect whole blood without anticoagulant. After allowing blood to clot, the serum should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20°C or lower.

Can I test cell culture supernatant in the Calcitonin ELISA?
Unfortunately, the Calcitonin ELISA has only been validated for use on human serum. We have no data to support the use on cell culture supernatant.

What should I do if the test sample is hemolyzed or lipemic?
Grossly hemolyzed, lipemic, and icteric samples as well as samples containing particulate matter or showing obvious microbial contamination should not be tested.

How long can samples be stored before testing?
Serum samples may be stored up to 8 hours at 2-8°C. Serum samples frozen at -20°C are stable for up to 4 months.

What is the minimum volume of sample required for testing?
For duplicate testing, 200µL of sample is required.

Can I test non-Human samples?
At this time, Biomerica has not validated its Calcitonin ELISA kit with the use of non-Human EDTA plasma or serum.

Reagents

Can I interchange lot numbers?
ELISA kit components are lot specific. Do not interchange lot numbers from previous kits.

What are the storage conditions for the kit components?
All reagents except the calibrators, kit controls and the Wash Concentrate are ready-to-use.
Store all reagents at 2-8°C.

How quickly should I pipette calibrators, controls and samples into the wells?
For each of the non-zero calibrators (Calibrator B through F) and kit controls 1 and 2, reconstitute each vial with 1.0 mL of Reagent 3 (Reconstitution Solution) and mix. Reconstitute Calibrator A with 2.0 mL of deionized or distilled water. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution.

Calcitonin 1-32 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls, and patient samples.

What is the stability of the Calibrators and Controls after reconstitution?
Use the calibrators and controls as soon as possible upon reconstitution. Freeze (-20°C) the remaining calibrators and controls as soon as possible after use.

Calibrators and controls are stable at -20°C for 6 weeks after reconstitution with up to 3 freeze thaw cycles.

What is the calibrator source in the Calcitonin ELISA?
The calibrator source in the calcitonin ELISA is synthetic human Calcitonin 1-32 in a buffer matrix. This kit does not

have any recombinant reagents.

What is the stability of the working wash solution?
The diluted working wash solution is stable for 90 days when stored at room temperature.

Is precipitate present in the wash concentrate normal?
Precipitate present in the Wash Concentrate is normal. This is due to storage at lower temperature such as 4°C. Dissolve by placing the vial in a 37° C water bath. If incubating the wash concentrate in a water bath does not dissolve the precipitate, the precipitate will dissolve once the wash concentrate is diluted to a 1X solution. To eliminate precipitate formation, the wash concentrate can be stored between 2-30°C according to the label.

ELISA Testing Procedure

What types of antibodies are used in the Calcitonin Test?
Calcitonin ELISA utilizes two different mouse monoclonal antibodies to human calcitonin specific for well-defined regions on the calcitonin molecule.

One antibody binds only to Calcitonin 11-23 and this antibody is biotinylated.

The other antibody binds only to Calcitonin 21-32 and this antibody is labeled with horseradish peroxidase [HRP] for detection.

What Precaution should I take when running this kit?
All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Common precautions in handling should be exercised, as applied to any untested patient sample.

Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

What is the maximum number of samples that can be tested on one kit?
If calibrators, controls, and samples are run in duplicate and two wells are left for the blank, a total number of **39** samples can be run on one ELISA kit.

What type of microplate shaker should be used when running this kit?
Biomerica has found for shaker diameters indicated below, the Calcitonin ELISA kit will maintain optimal performance response at the following speed settings:

Microplate Shakers	Shaking diameter	Speed (rpm) setting
Orbital	3 mm (0.1118 in)	600 ± 10 rpm
	19 mm (0.75 in)	170 ± 10 rpm
Linear	25 mm (0.98 in)	170 ± 10 rpm

Various types of shakers with different specifications are commercially available. In the event that the microplate shaker does not fall within the specified range above, each laboratory is encouraged to set its own optimal range.

Can I use an automated microplate washer when running the ELISA?
Yes, an automated plate washer may be used. If a microplate washer is unavailable, manual washing is also acceptable.

Can I run my assay via automated methods?
Biomerica has only validated its Calcitonin ELISA kit via manual testing as outlined in the approved IFU. You would need to perform your own validation to ensure equivalent performance between the manual method and the chosen automated method.

Can I run calibrators, controls, and samples in singlet?
It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.

Can I use incubation times and/or temperatures that are different than what are listed in the protocol?
Using incubation times and/or temperatures that differ from what is listed in the Package Insert may give erroneous results, and is therefore discouraged

Do I have to read the assay at both 450 and 405 nm?
Yes, the assay needs to be read at both 450nm and 405 nm. The 405 reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest calibrator, which is approximately 1,000 pg/mL.

Interpretation of Results

What concentration units are expressed in the test result?
The Biomerica Calcitonin ELISA expresses concentration results in pg/mL.

Curve Fit, QC, and Data Analysis

What type of curve fitting method is acceptable to interpret data results?
Computer programs using cubic spline or 4 PL [4 Parameter Logistics] can generally give a good fit.

What is considered an invalid Test?
In assays in which one or more of the quality control sample values lay outside the acceptable limits, the results for the patient sample should be considered invalid.

What is recommended for internal QC of each kit run?
It is recommended that control serum or serum pools should be evaluated with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods.

What should I do if the sample result is higher than the highest Calibrator?
Samples with Calcitonin levels greater than the highest calibrator, however, should be diluted and re-assayed for correct values. Dilute the sample in Calibrator A then re-assay. Ensure to multiply the results by the dilution factor.

Performance Characteristics

Which cross-reactants have been tested against Calicitonin (1-32)
Human PTH (1-84) at level up to 100,000 pg/mL showed 0.008% cross-reactivity. Calcitonin Gene Related Peptide at level up to 1,000,000 pg/mL showed 0.0002% cross-reactivity. Salmon Calcitonin at level up to 1,000,000 pg/mL showed 0.003% cross-reactivity. TSH (Thyroid-stimulating hormone) at level up to 5,000 uIU/ml showed 0.0006% cross-reactivity.

Does the Calcitonin ELISA have cross-reaction with Rat or other non-human species of Calcitonin?
Unfortunately, we don't have data to support cross-reactivity with rat or other non-human species. This assay has only been validated for use in human serum.

Are the Calcitonin Calibrators traceable to an international standard?
The calcitonin calibrators are traceable to WHO international standard NIBSC Code 89/620, human calcitonin.

Does this assay have a High Dose Hook Effect?
The Biomerica Calcitonin ELISA kit has exhibited no "high dose hook effect" with samples spiked with 1,000,000 pg/mL of pure intact calcitonin (1-32).

Can Heterophilic Antibodies in human serum react with kit reagents?
Samples from patients routinely exposed to animals or animal serum products may contain heterophilic antibodies that react with the reagent antibodies, potentially causing falsely elevated results. This assay has been formulated to mitigate the risk of this type of interference. However, potential interactions between patient sera and test components can occur.

Limitation of Procedure

What are some of the limitations of the Biomerica Calcitonin ELISA?
Like any analyte used as a diagnostic adjunct, Calcitonin results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

Supplemental Information

Can I purchase reagents individually?
The Calcitonin assay is intended to be sold as a complete kit. Please contact Biomerica for reagent availability

Troubleshooting Tips

Observation	Probable Cause
Low Absorbance	Improper calibrator/control preparation
	Enzyme or substrate reagent degradation
	Improper buffer/diluent used
	Reagents were cold when added to wells
	Incorrect incubation time
	Wells dried out
	Excessive wash/aspiration pressure from automated plate-washer
	Plate shaker not utilized
	Plate reader set at incorrect wavelength
High Background	Incomplete washing/aspiration of wells
	Substrate contamination
Poor Precision	Inaccurate pipetting
	Inadequate mixing of reagents
	Samples contain precipitates
	Wells dried out
	Contaminated well bottom
Poor Standard Curve	Improper dilution/handling of calibrators/controls
	Incomplete washing/aspiration of wells
	Inaccurate pipetting
	Improper diluent used for calibrator/control preparation

Calcitonin ELISA Kit – Procedural Flow Chart