Datasheet for ABIN956395

Calcitonin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Calcitonin (Calca)
Reactivity:	Dog
Method Type:	Sandwich ELISA
Detection Range:	7.8-500 pg/mL
Minimum Detection Limit:	7.8 pg/mL
Application:	ELISA

Product Details

Purpose:	This ELISA kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of dog CT in serum, plasma and other biological fluids.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of dog CT. No significant cross- reactivity or interference was observed.
Sensitivity:	The minimum detectable dose of dog CT is typically less than 3.84 pg/mL. The Sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by the mean O.D. value of 20 replicates of the zero calibrator plus three standard deviations.
Characteristics:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to CT. Calibrators and samples are then added to the appropriate microtiter plate wells with a biotin-

Product Details

	conjugated polyclonal antibody preparation specific for CT. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then the TMB substrate solution is added to each well. Only those wells that contain CT, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm +/- 2 nm. The concentration of CT in the samples is then determined by comparing the O.D. of the samples to the calibration curve.
Components:	Pre-coated, ready to use 96-well plate (1x) Calibrator (lyophilized) (2x) Calibrator Diluent (1 x 20 mL) Detection Reagent A (1 x 120 µL) Detection Reagent B (1 x 120 µL) Assay Diluent A (2X concentrate) (1 x 6 mL) Assay Diluent B (2X concentrate) (1 x 6 mL) TMB Substrate (1 x 9 mL) Stop Solution (1 x 6 mL) Wash Buffer (30X concentrate) (1 x 20 mL) Plate sealer for 96 wells (4x).
Material not included:	1. Microplate reader with 450 +/- 10 nm filter. 2. Precision single and multi-channel pipettes and disposable tips. 3. Eppendorf Tubes for diluting samples. 4. Deionized or distilled water. 5. Absorbent paper for blotting the microtiter plate. 6. Container for Wash Solution

Target Details

Target:	Calcitonin (Calca)
Alternative Name:	Calcitonin (CT) (Calca Products)

Application Details

Comment:	The calibration curve concentrations used for the ELISA's were 500, 250, 125, 62.5, 31.2, 15.6, 7.8 pg/mL.

Application Details

Plate:	Pre-coated
Assay Procedure:	Estimate the sample CT concentration before assaying. If the estimated values are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments. 1. Determine wells to be used for diluted calibrators, blank and samples. Prepare 7 wells for calibrators, 1 well for blank. Add 100 µL each of dilutions of calibrators (see Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37° C. 2. Remove the liquid from each well, do not wash. 3. Add 100 µL of the Detection Reagent A working solution to each well. Incubate for 1 hour at 37° C after covering with the Plate sealer. 4. Aspirate the solution and wash each well with 400 µL of 1X Wash Solution using a squirt bottle, multi- channel pipette, manifold dispenser or auto-washer, and let sit for 1~2 minutes. Remove the remaining liquid from all wells completely by sharply striking the plate on absorbent paper. Repeat wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. 5. Add 100 µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37° C after covering with the Plate sealer. 6. Repeat the aspiration/wash process five times as in step 4. 7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 25 minutes at 37° C (Do not exceed 30 minutes). Protect from light. The solution will turn blue after the addition of Substrate Solution. 8. Add 50 µL of Stop Solution to each well. The liquid will turn yellow after the addition of Stop solution. Mix the liquid by gently tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Remove any drops of solution or fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Then, run the microplate reader and conduct measurements at 450 nm immediately. Note: 1. Assay preparation: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Unused strips should be resealed and stored at 4° C until the expiration date. 2. Sample or reagent additions: Use freshly prepared Calibrators. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls if possible. For each step in the

Application Details

	procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all calibrators and samples, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each calibrator level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. After reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed. 4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drops of solution or fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too dark, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. 6. TMB Substrate is easily contaminated. Protect from light.
Calculation of Results:	Average the duplicate readings for each calibrator, control, and sample and subtract the average zero calibrator optical density. Create a calibration curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a calibration curve by plotting the mean absorbance for each calibrator on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CT concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. It is recommended to use some related software to do this calculation. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

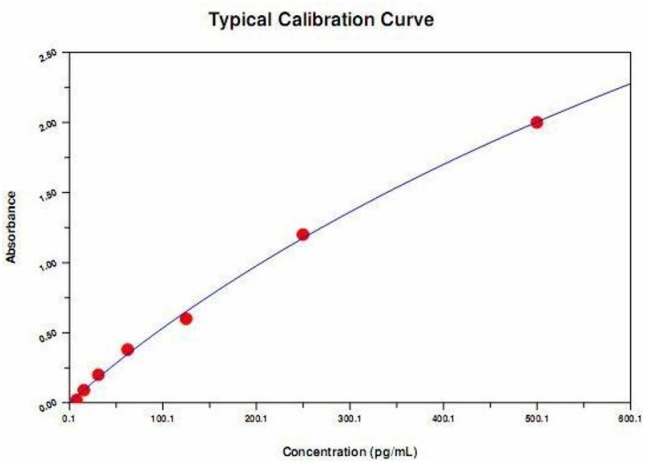
Handling

Storage:	-20 °C
Storage Comment:	All the reagents should be kept according to the labels on vials. The Calibrator, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20° C upon

Handling

	being received. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.
Expiry Date:	The expiry date is stated on the label.

Images



ELISA

Image 1.