Datasheet for ABIN956140

TNNC1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	TNNC1
Reactivity:	Dog
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Anti-cTnI-coated microtiter wells, 96 wells Dog cTnI Calibrator (lyophilized), reconstitute with 0.40 mL H2O cTnI Diluent, 12 mL cTnI HRP Conjugate, 11 mL Wash Solution (20X), 50 mL TMB Reagent (HRP substrate solution), 11 mL Stop Solution (1N HCl), 11 mL.
Material not included:	Pipettes: P-10, P-200 & P-1000 or equivalent Disposable pipette tips Distilled or de-ionized water Vortex mixer Absorbent paper

Product Details

	Graph paper or appropriate PC graphing software Polypropylene microcentrifuge tubes (1.5 mL) Microtiter plate reader capable of reading 0 to 4 OD at 450 nm.

Target Details

Target:	TNNC1
Alternative Name:	Cardiac Troponin-1 (TNNC1 Products)
Background:	Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. The troponin I subunit exists in three isoforms, two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. At the sequence level cardiac troponin-I (cTnI) is significantly different from the skeletal isoforms and antibodies can be prepared that specifically recognize cTnI. The unique isoform and tissue specificity of cTnI are the basis for its use as a marker of cardiac muscle damage

Application Details

Plate:	Pre-coated
Protocol:	The Dog Cardiac Troponin-I ELISA uses two different affinity purified cTnI specific antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horseradish peroxidase (HRP). The serum sample is allowed to react simultaneously with the two antibodies, resulting in cTnI being sandwiched between the solid phase and HRP-conjugated antibodies. After one hour incubation at room temperature on a plate shaker, the wells are washed to remove unbound HRP-conjugated antibodies. A solution of TMB (Tetramethylbenzidine), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of cTnI is proportional to the absorbance at 450 nm.
Sample Preparation:	Serum should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same). If serum samples cannot be assayed immediately they should be frozen at -70°C and thawed only once prior to use.
Assay Procedure:	1. Secure the desired number of coated wells in the holder. 2. Dispense 100 µL of cTnI HRP Conjugate into each well.

Application Details

	3. Dispense 100 µL of calibrators and samples into the appropriate wells. 4. Incubate at room temperature (18-25°C) on a plate shaker (150 rpm) for one hour. 5. Remove the incubation mixture either with a plate washer or by flicking plate contents into a bio-waste container. 6. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible (use of a plate washer gives optimal results). 7. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets. 8. Dispense 100 µL of TMB Reagent solution into each well. Gently mix for 5 seconds. 9. Incubate on a plate shaker (150 rpm) at room temperature for 20 minutes. 10. Stop the reaction by adding 100 µL of Stop Solution to each well. 11. Gently mix until all the blue color changes to yellow. 12. Read absorbance at 450 nm with a microtiter plate reader within 15 minutes. Please note: Due to plate reader differences, the high calibrator absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. 13. If absorbance values exceed the high calibrator, the samples should be appropriately diluted with cTnI diluent and re-determined. Samples with absorbance values below those of the lowest calibrator should be assigned a zero troponin-I value.
Calculation of Results:	1. Calculate the mean absorbance values (A450) for each set of reference calibrators and samples. 2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis. 3. Using the mean absorbance value for each sample, determine the corresponding concentration of cTnI in ng/mL from the calibration curve. 4. If available, graphing software may be used to analyze the data. Depending on the range of the calibration curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, calibration curves may be generated using a point-to-point fit.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	Store calibrator at or below -20°C. Store remainder of kit at 4°C. Keep the microtiter plate in a

Handling

	sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.
Expiry Date:	The expiry date is stated on the label.