Datasheet for ABIN956146

TNNC1 ELISA Kit

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Overview

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

Product Details

Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Anti-cTnI-coated microtiter wells, 96 wells Mouse cTnI Calibrator (lyophilized), reconstitute with 0.40 mL H2O Calibrator Diluent, 25 mL Plasma Diluent, 25 mL cTnI HRP Conjugate, 11 mL Wash Solution (20X), 50 mL TMB Reagent, 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

Product Details

	Absorbent paper 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 human troponin I subunit exists in three separate isoforms, two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnI) is about 40% dissimilar, has a molecular weight of 22,500 daltons, and has 31 additional amino acid residues that are not present on the skeletal isoforms. Antibodies made against the human cardiac isoform are immunologically different from antibodies made against the human skeletal isoforms, and the unique isoform and tissue specificity of cardiac troponin I is the basis for its use as an aid in the study of acute myocardial infarction (AMI) in humans.

Application Details

Plate:	Pre-coated
Protocol:	The high sensitivity Mouse Cardiac Troponin-I ELISA recognizes an epitope on mouse cTnI that is relatively resistant to proteolysis in mouse plasma, thereby improving detection capability. The assay uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horseradish peroxidase (HRP). The plasma sample is diluted with three volumes of plasma diluent and 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.

Application Details

Sample Preparation:	Plasma (EDTA) 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 for all samples). If plasma samples cannot be assayed within 4 hours of collection they should be frozen at -70°C and thawed only once prior to use. We recommend that samples be assayed in duplicate. Prior to assay, plasma samples should be diluted four fold with plasma diluent. This can easily be accomplished by mixing 100 µL of each plasma sample with 300 µL of plasma diluent in a polypropylene micro centrifuge tube.
Assay Procedure:	1. Secure the desired number of coated wells in the holder. 2. Dispense 100 µL of cTnI HRP Conjugate into each well. 3. Dispense 100 µL of calibrators and diluted samples into the appropriate wells. 4. Thoroughly mix. 5. Incubate at room temperature (18-25°C) on a plate shaker (150 rpm) for one hour. 6. Remove the incubation mixture by flicking plate contents into a waste container. 7. Wash and empty the microtiter wells 6 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. 8. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets. 9. Dispense 100 µL of TMB Reagent solution into each well. 10. Incubate on a plate shaker (~150 rpm) at room temperature for 20 minutes. 11. Stop the reaction by adding 100 µL of Stop Solution to each well. 12. Gently mix. It is important to make sure that all the blue color changes to yellow. 13. Read absorbance at 450 nm with a microtiter plate reader within 5 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. 14. If the absorbance values of the 4x diluted samples exceed those of highest calibrator, the 4x diluted plasma samples should be further diluted with calibrator diluent and re-tested (Do not use the plasma diluent for further dilution).
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. If using graphing software, we suggest using a point-to-point or a two site binding (hyperbola) fit of the data.

Application Details

	4. Multiply the derived cTnI concentrations by the dilution factor (i.e., 4, if the recommended dilution was used) to obtain the actual plasma cTnI concentration.
Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	The lyophilized reference calibrator should be stored at -20°C for optimum stability. The remainder of the kit should be stored refrigerated at 4°C. Keep the microtiter plate in a 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.