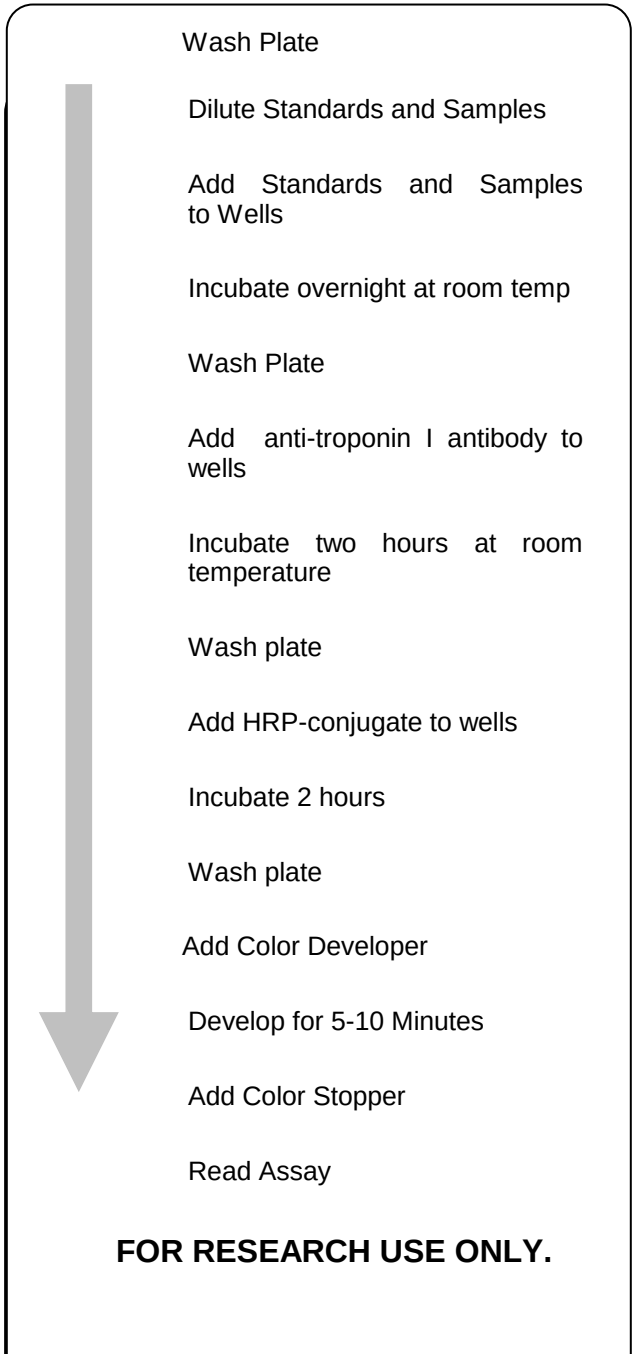
TROPONIN I M ELISA



FOR RESEARCH USE ONLY.

NOT FOR DIAGNOSTIC PROCEDURES

Troponin I M ELISA: An ELISA designed to measure murine Troponin I in plasma, serum or cell culture supertant.

Intended Use: Troponin I M ELISA is a direct ELISA for quantitation of murine Troponin I found in serum, plasma and supernatant. It is for research purposes, and is not intended for diagnostic use.

Technical Background: Troponin I is a complex of three proteins (troponin C, troponin I, and troponin T) that regulate skeletal and cardiac muscle contraction. Cardiac troponin I and T are sensitive and specific indicators of myocardial damage, and their blood levels rise in relation to the extent of injury to heart muscle from various conditions or from drug-induced cardiotoxicity. Cardiac troponins may be usefuls in predicting cardiovascular risk and mortality in the presence of chronic renal disease.
The mouse troponin I has three separate isoforms: two in skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnI) has a molecular weight of 22.5 kdaltons and is about 40% different from the skeletal isoforms. Antibodies specific for cardiac troponin I do not react immunologically with the other skeletal isoforms. The Exocell Troponin I M ELISA is a direct ELISA using two different affinity purified antibodies to troponin I. The first antibody is coated on a microtitre plate. The samples are added to the plate and incubated overnight. The plate is washed and then the anti-troponin I M antibody is added. This reaction is allowed to incubate for two hours at room temperature. The plate is washed and the HRP-conjugated antiobody is added for 2 hour. The plate is washed and the color developer is added for approximately 10 minutes. The murine Troponin I is then calculated from the standard curve.

Specimen Collection and Storage: Collect samples without preservative, separate and clarify them by centrifugation if necessary. Samples may be stored at 4˚C for up to 1 week or at -60˚C for up to 2 months. Prior to assay, allow the samples to come to room temperature. Do not apply heat to thaw frozen samples.

Kit Contents: The Troponin I M ELISA kit contains the following items:

a. 1 Troponin I Assay Plate
b. 2 NHEBSA Diluent
c. 2 Troponin I M Standard
d. 1 Anti-Troponin I M antibody
e. 1 HRP-conjugated antibody
f. 1 Color Developer
g. 1 Color Stopper
h. 1 Instructions

Specimen Required: Serum, Plasma or tissue extract.
Assay Range: 0.78 – 50.0 ng/ml
Precision: Intra- and inter-assay precision for samples within the assay range have a C.V. of <10%.

Materials Needed:
1. Wash Buffer-0.05% Tween20 in PBS, pH 7.2-7.4
2. ELISA plate reader capable of reading at 450 nm with reference at 630 nm. Can also be read at 450 nm.
3. Microfuge tubes for dilution of standard and samples.

Assay Procedure:
1. Bring all kit reagents to room temperature.
2. Serial dilutions of Troponin I Standard are made by adding 225 µl of NHEBSA Diluent to 6 tubes numbered 2 through 7. Standard #1 is 100ul taken straight from the standard tube of 50ng/ml. Standard #2, consists of removing 225 µl from the original standard vial and adding it to tube #2 and mix, remove 225 µl and add to tube #3 and mix. Continue with tubes #4 through #7. This will constitute a standard curve ranging from 50.0 ng/ml to 0.78 ng/ml.
3. Dilute samples to the appropriate concentration with NHEBSA Diluent, but at least 1:2 (undiluted samples should be avoided). A dilution series for unknowns of 1:5, 1:10 and 1:20 should be appropriate for most diabetic samples.
4. Aspirate each well and wash with wash buffer a total of five times by filling each well with wash buffer and removing the liquid, after the last wash remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
5. Add 100 µl of Troponin I Diluent to wells A1 and A2, this will be your negative control or blank wells. Add 100 µl of standard from stock vial to wells B1 and B2; add 100 µl from tube #2 to wells C1 and C2; add 100 µl from tube #3 to wells D1 and D2; add 100 µl from tube #4 to wells E1 and E2; add 100 µl from tube #5 to wells F1 and F2; add 100 µl from tube #6 to wells G1 and G2; and add 100 µl from tube #7 to wells H1 and H2.
6. Add 100 µl Sample to appropriate wells and record position on a plate map.
7. Incubate overnight at room temperature in a moist chamber.
8. Wash plate 6 times with wash buffer.
9. Add 100ul of anti-Troponin I M antibody to wells.
10. Incubate two hours room temperature in a moist chamber.
11. Wash plate six times with wash buffer.
12. Add 100 ul of HRP-conjugated antibody to wells.
13. Incubate two hour at room temperature in a moist chamber.
14. Wash the plate six times with wash buffer.
15. Add 100 µl of Color Developer to each well.
16. Allow blue color to develop for 5-20 minutes.
17. Add 100 µl of Stop Solution to each well.
18. Read in ELISA plate reader at 450 nm. Reference blank is at 630 nm.

Calculation of Results:

Average the duplicate readings for each standard and sample and subtract the average zero (blank) optical density. Create a standard curve using computer software capable of a four parameter logistic curve fit. As an alternative, construct a standard curve by plotting the absorbance against the concentration of Troponin I M and draw a best fit curve. The data may be linearized by plotting the log absorbance against the log concentration and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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