



cTnI ELISA

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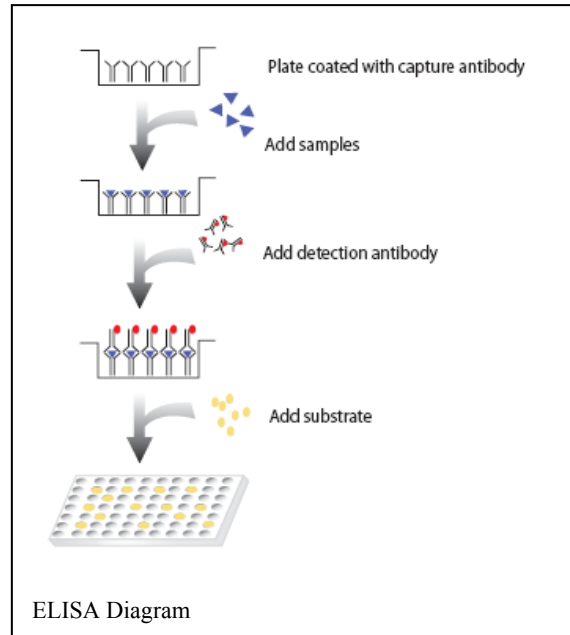
Introduction

Troponin, a complex of three proteins (troponins T, I, and C) that bind to the thin filament (actin) of striated muscle, regulates muscle contraction via calcium binding (1). Following injury to muscle cells (heart or skeletal muscles) the intact troponin complex along with free troponin subunits are released into blood. Troponin I subunit exists in three separate isoforms; two in skeletal muscle, and one in cardiac muscle (2). Because of its tissue specificity, cardiac troponin I (cTnI) has been useful in the differential diagnosis of patients presenting to Emergency Departments (ED) with chest pain and becomes the most preferred biomarker for the use in the diagnosis of acute myocardial infarction (AMI) (3). Like CK-MB, cardiac TnI has similar release patterns (4-6 hours after the onset of pain and a peak in approximately 12 to 24 hours after infarction), but the level of cTnI remains elevated for a much longer period of time (6-10 days), thus providing for a longer window of detection of cardiac injury (4).

The Signosis cTnI Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of cardiac-specific troponin I. The antibodies developed for the test will determine a minimal concentration of 1.0 ng/ml, and there is no cross-reactivity with human cardiac troponin T or skeletal troponin T or I.

Principle of the assay

The cTnI ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes three mouse monoclonal antibodies against distinct determinants on cTnI for immobilization on the microtiter wells and a mouse anti-cTnI monoclonal antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in cTnI being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of cTnI is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kit

- Antibody-Coated Wells (1 plate, 96 wells) Microtiter wells coated with mouse monoclonal anti-cTnI.
- Reference Standard Set (1 set, 1.0 ml/vial) Contains 0, 2.0, 7.5, 30, and 75 ng/ml cTnI, lyophilized.
- cTnI Enzyme Conjugate Reagent (13 ml/vial) Contains mouse monoclonal anti-TnI conjugated to horseradish peroxidase in Tris Buffer-BSA solution with preservatives.
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Warning and precautions

1. Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (5) or other appropriate national biohazard safety guidelines or regulations (6-8).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

Reagent preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.

Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8°C.

Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (-20 °C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

Assay procedure

1. Add 100µl of standards, specimens, and controls into appropriate wells.
2. Add 100µl of Enzyme Conjugate Reagent into each well.
3. Thoroughly mix for 30 seconds. It is very important to mix them completely.
4. Incubate at room temperature (18-25°C) for 90 minutes.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Add 100µl of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

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Example of standard curve

cTnl (ng/ml)	Absorbance (450 nm)
0	0.048
2.0	0.110
7.5	0.307
30	1.357
75	2.853

