



AccuDiag™ Troponin I ELISA Kit

REF 1105-18

IVD See External Label 2°C 8°C 96 Tests

Troponin I ELISA

Method	Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-75 ng/ml
Sample	100 µL serum
Incubation Time	110 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- ✓ Very easy to use with little training
- ✓ Highly specific and consistent Assay
- ✓ Provides accurate results quickly
- ✓ Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation, Inc. (DAI) cTnI ELISA is intended for the quantitative determination of cardiac-specific troponin I in human serum.

ASSAY PRINCIPLE

The cTnI ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes four unique monoclonal antibodies directed against distinct antigenic determinants on the molecule. Three mouse monoclonal anti-troponin I antibodies are used for solid phase immobilization (on the microtiter wells). The fourth antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin I molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 90-minute incubation at room temperature, the wells are

washed with water to remove unbound-labeled antibodies. A solution of tetramethylbenzidine (TMB) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of troponin I is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the test kit

- Antibody-Coated Wells (1 plate, 96 wells)**
Microtiter wells coated with mouse monoclonal anti-TnI.
- Reference Standard Set (1 set, 1.0 ml/vial)**
Contains 0, 2.0, 7.5, 30, and 75 ng/ml TnI, 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 (11 ml/bottle)**
Contains one-step TMB solution.
- Stop Solution (11 ml/bottle)**
Contains diluted hydrochloric acid (1N HCl).

Materials required but not provided

- Distilled or deionized water
- Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper
- Cardiac Marker Plus Tri Liq Controls, Cat. No. 180 (Bio-Rad Laboratories Diagnostics Group, Hercules, CA 94547)

Instrumentation

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

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 21 days when stored sealed at 2-8°C. Discard the reconstituted Standards after 21 days. To assure long term (more than 21 days) 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.**
- Samples with expected Troponin I concentrations over 75 ng/ml may be quantitated by dilution with diluent available from vender.

PRECAUTIONS

- 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 samples be handled according to the

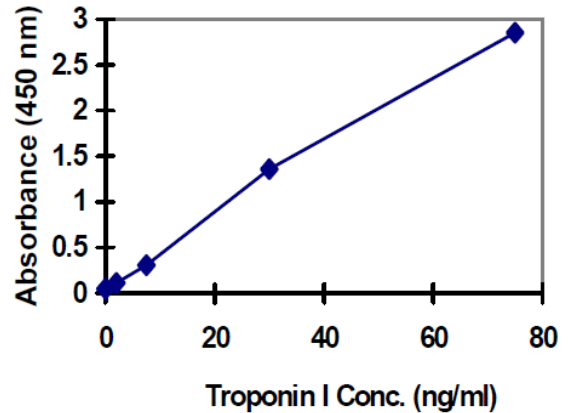


OSHA Standard on Bloodborne Pathogens or other appropriate national biohazard safety guidelines or regulations.

- 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.
- Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
- Replace caps on reagents immediately. Do not switch caps.
- Do not pipette reagents by mouth.

A. Example of standard Curve:

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



ASSAY PROCEDURE

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

RESULTS

- Calculate the mean absorbance value (OD₄₅₀) for each set of reference standards, controls and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of troponin I (ng/ml) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
- Samples with cTnI concentrations greater than 75 ng/ml should be diluted 10-fold with vender's Troponin I Sample Diluent. The final cTnI values should be multiplied by 10 to obtain cTnI results in ng/ml.

EXAMPLE OF A STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against troponin I concentrations shown on the X axis. **NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

STORAGE

- Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
- Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	2022-09
REF 1105-18	AccuDiag™ - Troponin I ELISA
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EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
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