

Troponin I ELISA

Quantitative Immunoenzymatic determination of Troponin I in human serum

for research use only

RUO



LOT

See external label

2°C  8°C



Σ = 96 tests

REF DKO180

INTENDED USE

Eagle Biosciences [Troponin I ELISA Assay Kit](#) is an immunoenzymatic colorimetric method for quantitative determination of Troponin I concentration in human serum. Troponin I ELISA Assay Kit is intended for research use only and not intended for diagnostic procedures.

1. CLINICAL SIGNIFICANCE

Troponin I is a 24kDa protein and is part of a complex, called Troponin complex, that regulates the calcium-modulate interaction between actin and myosin in the striated muscle; this complex is an heterodimer consisting of Troponin C, I and T; Troponin I is the inhibitory subunit of this complex.

Troponin complex is tightly bound to the contractile apparatus, but many studies shows that the Troponin I is released into the blood circulation in case of myocardial damage, such as in AMI (Acute Myocardial Infarction), where the myofibril is seriously damaged. The presence of Troponin I in the circulation can be detected for many days after AMI, thus the detection of Troponin levels in blood fluids is an excellent biomarker for myocardial damage.

It should be note that in case of marathon runners or other skeletal injury the Troponin I has not been found in circulation, thus it is a very specific marker for AMI.

2. PRINCIPLE

In the first step the serum sample and the Conjugate are added to the microplate coated with Streptavidin. The conjugate in this Troponin I ELISA Assay Kit contains two monoclonal antibodies direct against different epitopes of Troponin I: one is biotinylated, the other one is bound to the horseradish peroxidase (HRP); these antibodies compete for the native Troponin I in the sample, thus at the end of first step a sandwich between the antibodies and the Troponin I is formed; this complex binds to the microplate wells through the specific interaction between biotine and streptavidin.

At the end of incubation, the bound/free separation is performed by a simple solid-phase washing. In the next step, the enzyme HRP in the bound-fraction reacts with the Substrate and develops a blue color; the reaction is stopped by adding the Stop Solution. The concentration of Troponin I in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

- Calibrators (6 vials, lyophilized)
CAL0 **REF** DCE002/18006-0
CAL1 **REF** DCE002/18007-0
CAL2 **REF** DCE002/18008-0
CAL3 **REF** DCE002/18009-0
CAL4 **REF** DCE002/18010-0
CAL5 **REF** DCE002/18011-0
Reconstitute each vial with 1 mL of distilled or deionized water.
- Conjugate (1 vial, 13 mL, ready to use)
Monoclonal biotinylated anti Troponin I antibody and monoclonal anti Troponin I antibody conjugated with horseradish peroxidase (HRP) **REF** DCE002/18002-0
- Coated Microplate (1 breakable microplate)
Microplate coated with Streptavidin **REF** DCE002/18003-0
- Substrate A (1 vial, 7 mL)
Contains tetramethylbenzidine (TMB) (*avoid any skin contact*) **REF** DCE004/18004-0
- Substrate B (1 vial, 7 mL)
Contains H₂O₂ (*avoid any skin contact*) **REF** DCE004/18004-0
- Stop Solution (1 vial, 8 mL)
Sulphuric acid 0.15 mol/L (*avoid any skin contact*) **REF** DCE005/18005-0
- 50X Conc. Wash Solution (1 vial, 20 mL)
Phosphate buffer **REF** DCE006/18006-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm, 620-630 nm)

Note

Store all reagents between 2-8°C in the dark.

Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS

- This Troponin I ELISA Assay Kit is intended for research use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.

- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- Some reagents in the Troponin I ELISA Assay Kit contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents in the Troponin I ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all Troponin I ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange Troponin I ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.

- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Working Substrate

Put the content of the Substrate A into the bottle of Substrate B. Mix well. The reagent is now ready to be used during the assay.
The Substrate is stable for 2 months, store at 2-8°C.

6.2. Preparation of the Calibrators (C₀...C₅)

The Calibrators are lyophilized and are calibrated against NIST standards for Troponin I # 2921; they have the following concentrations:

	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
ng/mL	0	1	3	6	15	30

Reconstitute each vial with 1 mL of distilled or deionized water; reconstitute Calibrators are stable for 24 hours at 2-8°C; for longer storage aliquot the Calibrators and store at -20°C (do not freeze and thaw more than once).

Samples with Troponin I concentration higher than 30 ng/mL should be diluted with the Calibrator 0 or with a serum Troponin I-free; consider this dilution in the final calculation of the original sample.

6.3. Preparation of the Sample

The determination of Troponin I with Eagle Biosciences Troponin I kit should be performed in human serum.

The samples can be stored at 2-8°C for 5 days; for longer storage up to 30 days store at -20°C (avoid repetitive freezing and thawing).

6.4. Preparation of Wash Solution

Dilute the contents of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₅), two for each sample, one for Blank.

Reagent	Calibrator	Sample	Blank
Calibrator C ₀ -C ₅	25 µL		
Sample		25 µL	
Conjugate	100 µL	100 µL	
Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap. Incubate for 15 minutes at room temperature (22-28°C). Remove the contents from each well; wash the wells 3 times with 350 µL of diluted Wash Solution. Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
Substrate	100 µL	100 µL	100 µL
Do not shake after substrate addition. Incubate at room temperature (22±28°C) for 15 minutes in the dark.			
Stop Solution	50 µL	50 µL	50 µL
Shake the microplate gently for 15-20 seconds. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Troponin I for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C₀-C₅) and of each sample.

8.2. Calibration curve

Plot the values of absorbance (Em) of the Calibrators (C₀-C₅) against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

8.4. Validation of Results

The following criteria should be met:

Calibrator	Absorbance (OD)
Cal 0	OD ≤ 0.070
Cal 5	OD ≥ 1.300

Please refer always to the Certificate of Analysis lot-specific.

9. EXPECTED VALUES

The following range obtained by clinical data in concordance with the published literature should be used as a guidelines only:

	Troponin I value
Adult – normal	≤ 1.3 ng/mL

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate the measurement (20x) of three different control sera in one assay. The within assay variability is ≤ 3.3%.

10.1.2. Inter Assay Variation

Between run variation was determined by replicate the measurement (10x) of three different control sera in different lots of kit. The between assay variability is ≤ 7.9%.

10.2. Sensitivity

The lowest detectable concentration of Troponin I that can be distinguished from the Calibrator 0 is 0.03 ng/mL at the 95% confidence limit.

10.3. Specificity

The cross reaction was evaluated by adding the interfering substances to a serum sample at a very high concentration:

Substance	Cross reactivity
Hemoglobin	ND
CK-MB	ND
TnT	ND
FABP	ND

10.4. Correlation with RIA

Eagle Biosciences Troponin I kit was compared to a radioimmunoassay kit. Serum samples from 151 symptomatic and asymptomatic persons were analysed according to both test systems.

The linear regression curve was calculated:

$$y = 0.9266 x + 0.35$$

$$r^2 = 0.950$$

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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DiaMetra S.r.l. Headquarter: Via Garibaldi, 18 –
20090 SEGRATE (MI) Italy
Tel. 0039-02-2139184 – 02-26921595
Fax 0039-02-2133354.

Manufactory: Via Pozzuolo 14, 06034 SPELLO (PG)
Italy
Tel. 0039-0742-24851
Fax 0039-0742-316197
E-mail: info@diametra.com

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110

www.EagleBio.com • info@eaglebio.com



