Jan 16, 2026

Standard Operating Procedure (SOP): Mouse Troponin T Type 2 (cardiac) ELISA Kit (Colorimetric)

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwnd1dvmk/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwnd1dvmk/v1

Protocol Citation: Elizabeth Rocker 2026. Standard Operating Procedure (SOP): Mouse Troponin T Type 2 (cardiac) ELISA Kit (Colorimetric). **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwnd1dvmk/v1

Protocol status: In development
We are still developing and optimizing this protocol

Created: January 14, 2026

Last Modified: January 16, 2026

Protocol Integer ID: 238638

Keywords: troponin isoform, troponin complex, tropomyosin, biological marker for cardiomyocyte, indicator of myocardial cell, myocardial cell, biological marker, cardiac, cardiomyocyte, calcium sensitivity, elisa kit, contractile apparatus in muscle

Abstract

Troponin T is one of three troponin isoforms found in the tropomyosin-troponin complex. This complex is responsible for the calcium sensitivity of the contractile apparatus in muscle. Cardiac Troponin T is used as a biological marker for cardiomyocytes and its level in serum is frequently used as an indicator of myocardial cell injury.

Administrative

1 **Version:**
1.0
Effective
Date: 01/05/2026
Approved
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Prepared
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Sample Preparation

2 Dilute the experimental samples (serum, plasma, lysates or other biofluids) and perform a
serial dilution of the standard (purified form of the analyte under consideration) in Reagent Diluent. If available, use the literature to determine the optimal concentration and linear working range. Samples should be prepared in duplicate, or preferably in triplicate.

Procedure

3 Use an ELISA compatible PVC clear microtiter plate (96-well) and determine the number of wells needed for the assay.

4 Dispense 100 µL of Capture Antibody Solution into the wells. Apply sealing tape to the top of the plate to prevent evaporation. Incubate the plate overnight at 4 °C. After incubation remove the tape and aspirate each well.

5 Dispense 400 µL of Wash Buffer into each well. Then invert the plate between washes and flick out the solution. Wash the plate 2 more times and pat the inverted plate on a paper towel to dry.

6 Block plates by dispensing 300 µL of Reagent Diluent into each well and incubate the plate for 60 minutes at room temperature. After incubation, remove the tape. Invert the plate and flick out the solution.

7 Repeat step 3.

8 Add 100 µL of diluted samples and standards to the appropriate wells and cover the plate.

9 Incubate the plate for 2 hours at room temperature. After incubation, uncover the plate and aspirate each well.

10 Repeat step 3.

11 Dispense 100 µL of Detection Antibody Solution to each well. Incubate for 2 hours at room temperature and cover the plate. After incubation, uncover the plate. Invert the plate and flick out the solution.

12 Repeat step 3.

13 Dispense 100 µL of SA Solution into each well. Incubate for 20 minutes at room temperature and cover the plate. After incubation, uncover the plate. Invert the plate and flick out the solution.

14 Repeat step 3.

15 Dispense 100 µL of the Substrate Solution into each well. Cover the plate and incubate for to 30 minutes at room temperature. Avoid placing the plate in direct sunlight.
TIP: If no color develops within 30 minutes, incubation time can be extended until an appropriate color change/signal is acquired. TMB substrate reacts with HRP to generate a blue colored product which changes to yellow upon the addition of Stop Solution, whereas the PNPP substrate reacts with AP to generate a yellow-colored product.

16 After color development, remove the cover and dispense 50 µL of Stop Solution into each well to stop the enzymatic reaction.

17 Immediately measure the absorbance of each well using a spectrophotometer/plate reader with the appropriate absorbance setting.
TIP: The presence of air bubbles in wells may impact the results and it is advisable to spin the plate in a centrifuge.