

Certificate of Analysis

Cardiac Troponin I

(Recombinant protein expressed in Sf21 insect cells)

Item # 23-055, 23-055-K, 23-055M

Parent Lot # D12BP011N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal c-Myc, GST-tagged, recombinant human Cardiac Troponin I full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 68% by SDS-PAGE and Coomassie blue staining. MW = 52kDa.

Formulation: 0.399mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

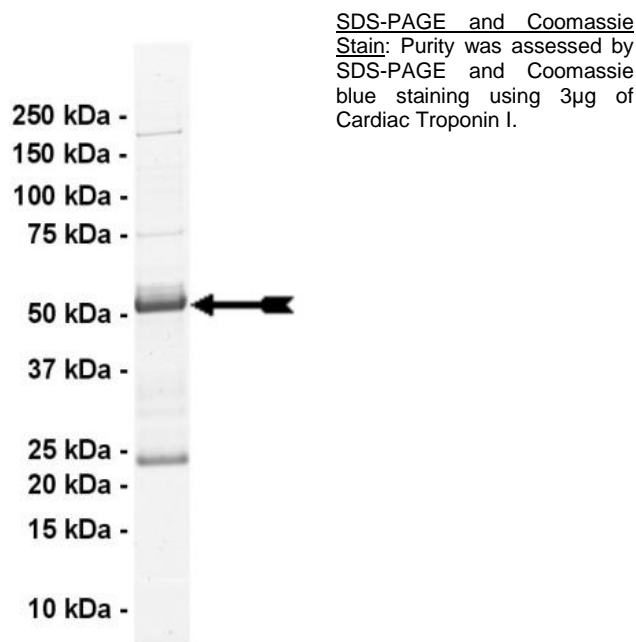
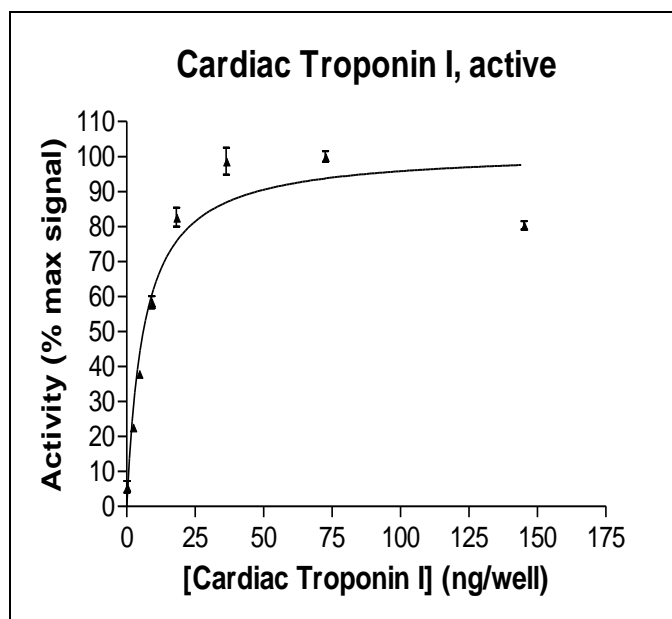
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

Protein Identity: Confirmed identity as Cardiac Troponin I by mass spectrometry



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Assay Protocol

Reagents:

- | | |
|-----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021) | 4. 1x Reaction Buffer |
| 2. Ubch5c, active (Item # 23-035) | 5. Biotinylated-Ubiquitin |
| 3. MuRF1, active (Item # 23-054) | 6. Stop Solution |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂).

Cardiac Troponin I is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, 10nM UBE1, 500nM Ubch5c, 0.02μg MuRF1, and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of cardiac troponin I is measured by detection of bound ubiquitin via electrochemiluminescence.

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Cardiac Troponin I Information

Protein human Cardiac Troponin I

Accession number GenBank NM_000363

Alternative Names Troponin I, cardiac muscle, TNNI3, TNNC1

Key Facts Troponin is a complex of three regulatory proteins (Troponin I, Troponin T and Troponin C). It is located on the actin filament and is essential for the calcium-mediated regulation of skeletal and cardiac muscle contraction. There are tissue-specific isoforms of Troponin I, T and C and two subtypes (Cardiac Troponin I and T) are released when the heart muscle has been damaged, such as during a heart attack. The more damage there is to the heart, the greater the amount of Troponin I and T there will be in the blood and currently, Cardiac Troponin I and T are used as the preferred biochemical markers for the diagnosis of myocardial infarction. It has also been demonstrated that Cardiac Troponin I is targeted for degradation by MuRF1, an E3 ubiquitin ligase that has been linked to muscle atrophy

Related Products Item # 23-021 UBE1, active, Item # 23-035 Ubch5c, active, Item # 23-054 MuRF1, active

Selected References

Babuin L. and Jaffe A. S. Troponin: the Biomarker of Choice for the Detection of Cardiac Injury. CMAJ, 173: 1191-1202, 2005

Korff S. *et al.*, Differential Diagnosis of Elevated Troponins. Heart, 92: 987-993, 2006

Kedar V. *et al.*, Muscle-Specific RING Finger 1 is a Bona Fide Ubiquitin Ligase that Degrades Cardiac Troponin I. Proc Natl Acad Sci U S A., 101: 18135-18140, 2004

Reviewed and approved by site quality representative.

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