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Certificate of Analysis

MuRF1, active

(Recombinant E3 ligase expressed in *E.coli*)
Item # 23-054, 23-054-K, 23-054M
Parent Lot # D11SP001N

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 GST-tagged, recombinant human MuRF1 full length, expressed in *E.coli*. Purified using glutathione sepharose.
Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 67kDa.

Activity (Parent lot# D11SP001N): This lot of MuRF1, active is active and meets product specifications.

Formulation: 0.285mg/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.

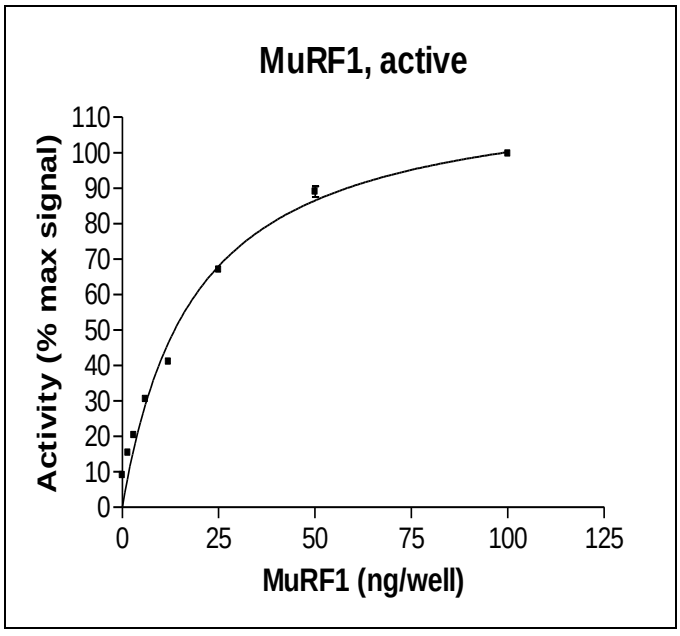
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

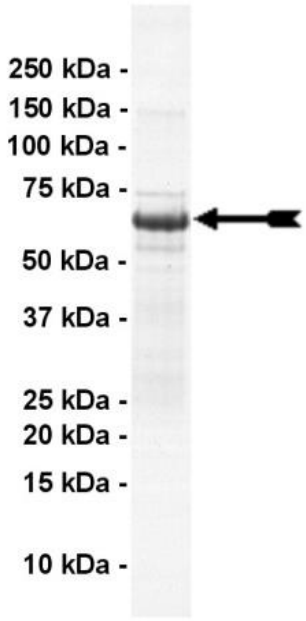
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 MuRF1 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MuRF1, active.

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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH5c, active (Item # 23-035)**
3. **MuRF1, active (Item # 23-054)**
4. **Cardiac Troponin I (Item # 23-055)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

MuRF1 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 500nM UbcH5c, 100nM Cardiac Troponin I, 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. MuRF1 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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MuRF1 Information

Protein human MuRF1

Accession number GenBank NM_032588

Alternative Names E3 ubiquitin-protein ligase TRIM63, Iris RING finger protein (IRF), Muscle-specific RING finger protein 1, RING finger protein 28 (RNF28), Striated muscle RING zinc finger protein (SMRZ)

Key Facts Muscle atrophy is a debilitating and life threatening disorder. It is associated with a number of diseases including cancer, diabetes, chronic obstructive pulmonary disease and AIDS and is a natural consequence of inactivity and aging. MuRF1 is an E3 ubiquitin ligase which has been implicated as a component of atrophy-associated proteolysis. It contains an amino terminal RING finger that mediates ubiquitin transfer along with a zinc finger and two coiled-coil domains in its central region. It is up-regulated in a number of different models of atrophy and interacts with numerous myofibrillar proteins as well as Cardiac Troponin I and Creatine Kinase. Deletion of MuRF1 in mice has been shown to inhibit skeletal muscle atrophy suggesting that it may be an important drug target for the prevention of muscle wasting.

Related Products Item # 23-021 UBE1, active, Item # 23-035 UbcH5c, active, Item # 23-055 Cardiac Troponin I

Selected References

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Foletta V. C. *et al*., The Role and Regulation of MAFbx/atrogin-1 and MuRF1 in Skeletal Muscle Atrophy. Pflugers Arch. *461*: 325-335, 2011

Koyama S. *et al.,* Muscle RING-Finger Protein-1 (MuRF1) as a Connector of Muscle Energy Metabolism and Protein Synthesis. J Mol Biol., *376*: 1224-1236, 2008

Polge C. *et a*l., Muscle Actin is Polyubiquitinylated *in vitro* and *in vivo* and Targeted for Breakdown by the E3 Ligase MuRF1. FASEB J., *25*: 3790-3802, 2011

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.