MuRF1 [untagged]

E3 Ligase

Alternate Names: TRIM63, Ring Finger protein 28, RNF28, Striated Muscle Ring Zinc finger protein, SMRZ

Cat. No. **63-0047-025**
Lot. No. **30268**

Quantity: 25 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Muscle Ring Finger protein 1 (MuRF1) is a member of the E3 protein ligase family and cloning of the human gene was first described by Dai and Liew *et al.* (2001). Muscle atrophy is a pathological condition associated with a number of diseases including cancer, metabolic disorders and AIDS. The associated up-regulation of MuRF1 and increased muscle protein turnover observed in disease models highlights the potential value of targeting this E3 ligase in order to address muscle wasting conditions. Atrophy of skeletal muscle can occur through lack of use and denervation associated with disease states such as cancer cachexia, cardiac failure and other muscle wasting conditions in humans (Cohen *et al.*, 2009; Rottbauer *et al.*, 2006; Seidman *et al.*, 2001; Poetter *et al.*, 1996). Myosin Light Chain 1 (MyLC1) and MyLC2 are required for thick-filament stabilization and normal contractility in the myofibrils of skeletal muscle. Loss of function mutations in these proteins results in a perturbation of contractility and a striking disorganization of the thick filaments which leads to muscle wasting conditions (Cohen *et al.*, 2009; Seidman *et al.*, 2001). Rapid loss in muscle mass due to protein degrada-

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 25 µg

Concentration: 0.25 mg/ml

Formulation: 50 mM sodium bicarbonate pH 10, 250 mM NaCl, 0.03% Brij35, 0.5 mM TCEP

Molecular Weight: ~40.2 kDa

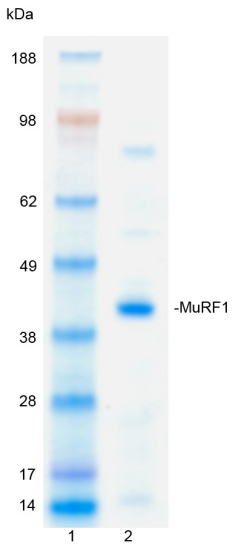
Purity: >50% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

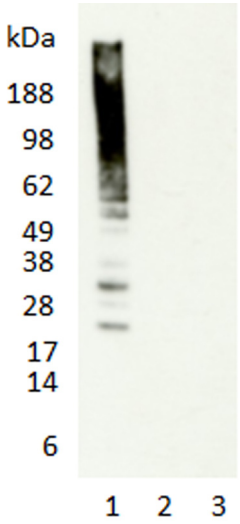
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg MuRF1



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of MuRF1 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of MuRF1 for 60 minutes at 30oC in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or MuRF1 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and MuRF1.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

MuRF1 [untagged]

E3 Ligase

Alternate Names: TRIM63, Ring Finger protein 28, RNF28, Striated Muscle Ring Zinc finger protein, SMRZ

Cat. No.	**63-0047-025**	Quantity:	25 µg
Lot. No.	**30268**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

Continued from page 1

tion of the myofibrilar components is mediated by the ubiquitin proteasome pathway. During this process there is a marked increase in MuRF1 concentration which is thought to act directly on specific components of the myofibril to promote its disassembly and degradation during atrophy (Cohen *et al.*, 2009). Mice lacking the RING domain of MuRF1 show significantly reduced loss of muscle mass following denervation highlighting the therapeutic importance of this target in muscle wasting disease (Cohen *et al.*, 2009).

References:

Cohen S, Brault JJ, Gygi SP, Glass DJ, Valenzuela DM, Gartner C, Latres E, Goldberg AL (2009) During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation. *J Cell Biol* **185**, 1083-95.

Dai KS, Liew CC (2001) A novel human striated muscle RING zinc finger protein, SMRZ, interacts with SMT3b via its RING domain. *J Biol Chem* **276**, 23992-23999.

Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, Rayment I, Sellers JR, Fananapazir L, Epstein ND (1996) Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* **13**, 63-9.

Rottbauer W, Wessels G, Dahme T, Just S, Trano N, Hassel D, Burns C, Katus H, Fishman M (2006) Cardiac myosin light chain-2: a novel essential component of thick-myofilament assembly and contractility of the heart. *Circ Res* **99**, 323–331.

Seidman JG, Seidman C (2001) The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell* **104**, 557-67.

Physical Characteristics

Continued from page 1

Protein Sequence:

MDYKSSLIQDGNPMENLEKQLICPICLEM
FTKPVVILPCQHNLCRKCANDIFQAAN
PYWTSRGSSVSMSGGRFRCPTCRHEVIMDRH
GVYGLQRNLLVENIIDIYKQECSSRPLQKG
SHPMCKEHEDEKINIYCLTCEVPTC
SMCKVFGIHKACEVAPLQSVFQGQK
TELNNCISMLVAGNDRVQTIITQLEDSR
RVTKENSHQVKEELSQKFDTLYAILDEKK
SELLQRITQEQEKKLSFIEALIQQY
QEQLDKSTKLVETAIQSLDEPGGAT
FLLTAKQLIKSIVEASKGCQLGKTEQG
FENMDFFTLDLEHIADALRAIDFGT
DEEEEEFIEEEDQEEEESTEGKEEGHQ

MuRF1 (regular text): Start ***bold italics*** (amino acid residues 1-353)
Accession number: NP_115977.2

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0