

TRIAD1 [6His-tagged]

E3 Ligase

Alternate Names: Triad domain-containing protein 1; *Drosophila Ariadne* homolog 2; ARIH2

Cat. No. 63-0029-025

Lot. No. 30026

Quantity: 25 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 1

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). Triad Domain-Containing Protein 1 (TRIAD1) is a member of the E3 protein ligase family and cloning of the human gene was first described by van der Reijden *et al.* (1999). TRIAD1 contains a TRIAD motif containing two RING domains which flank a conserved cysteine-rich (C6HC) domain designated DRIL (double RING finger-linked domain) (Marteijn *et al.*, 2005). TRIAD is thought to be involved in protein translation, interacting with Ubch7 (UBE2L3) to polyubiquitylate eIF4E2 targeting it for proteasomal degradation (Tan *et al.*, 2003). More recently these proteins have been referred to as Ring in-between Ring E3 ligases (RBRs) that function like RING-HECT hybrids regulating processes such as translation and immune signaling (Wenzel *et al.*, 2011).

References:

Marteijn JA, van Ernst L, Erpelinck-Verschueren CA, Nikoloski G, Menke A, de Witte T, Lowenberg B, Jansen JH, van der Reijden BA (2005) The E3 ubiquitin-protein ligase Triad1 inhibits clonogenic growth of primary myeloid progenitor cells. *Blood* 106, 4114-23.

Tan NG, Ardley HC, Scott GB, Rose SA, Markham AF, Robinson PA (2003) Human homologue of ariadne promotes the ubiquitylation of translation initiation factor 4E homologous protein, 4EHP. *FEBS Lett* 554, 501-4.

van der Reijden BA, Erpelinck-Verschueren CA, Lowenberg B, Jansen JH (1999) TRIADs: a new class of proteins with a novel cysteine-rich signature. *Protein Sci* 8, 1557-61.

Wenzel DM, Lissounov A, Brzovic PS, Klevit RE (2011) UBCH7 reactivity profile reveals parkin and HHAR1 to be RING/HECT hybrids. *Nature* 474, 105-8.

Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~61.2 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

MSY YHHHHHHDYDIPTTENLYFQ G A M G S M S
V D M N S Q G S D S N E E D Y D P N C E E E E E E E E E D
D P G D I E D Y Y V G V A S D V E Q Q G A D A F D P E E Y Q F T C L
T Y K E S E G A L N E H M T S L A S V L K V S H S V A K L I L V N F H
W Q V S E I L D R Y K S N S A Q L L V E A R V Q P N P S K H V P T S H P
P H H C A V C M Q F V R K E N L L S L A C Q H Q F C R S C W E Q H C S V
L V K D G V G V G V S C M A Q D C P L R T P E D F V F P L L P N E E L
R E K Y R R Y L F R D Y V E S H Y Q L Q L C P G A D C P M V I R V Q E
P R A R R V Q C N R C N E V F C F K C R Q M Y H A P T D C A T I R K
W L T K C A D D S E T A N Y I S A H T K D C P K C N I C I E K N G
G C N H M Q C S K C K H D F C W M C L G D W K T H G S E Y Y E C S
R Y K E N P D I V N Q S Q Q A Q A R E A L K K Y L F Y F E R W E N H N K
S L Q L E A Q T Y Q R I H E K I Q E R V M N N L G T W I D W Q Y L Q
N A A K L L A K C R Y T L Q Y T Y P Y A Y Y M E S G P R K K L F E Y
Q Q A Q L E A E I E N L S W K V E R A D S Y D R G D L E N Q M H I
A E Q R R R T L L K D F H D T

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYF▼QG)
TRIAD1 (regular text): Start **bold italics** (amino acid residues 1-493)
Accession number: NP_006312

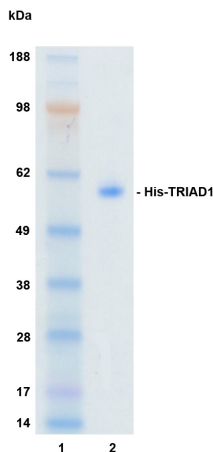
Quality Assurance

Protein Identification:

Confirmed by mass spectrometry.

Purity:

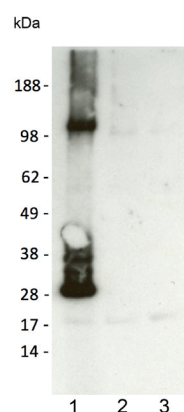
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-TRIAD1



E3 ligase assay:

The ubiquitin conjugating activity of His-TRIAD1 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-UBE2D1 (Ubch5a) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of His-

TRIAD1 for 120 minutes at 37°C in the presence of ubiquitin, His-UBE1, His-UBE2D1 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or His-TRIAD1 (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 His-TRIAD1.



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