TRIAD1 [6His-tagged]

E3 Ligase

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

Cat. No.	**63-0029-025**	Quantity:	25 µg
Lot. No.	**30026**	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 signalling (Wenzel *et al.*, 2011).

References:

Marteijn JA, van Emst 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 HHARI 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:

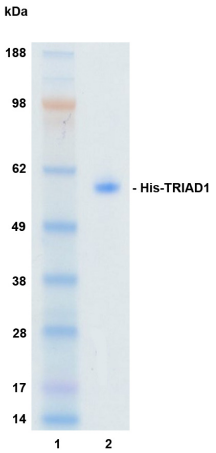
MSYYHHHHHHDYDIPTTENLYFQGAMGS***M***S
VDMNSQGSDSNEEDYDPNCEEEEEEEEED
DPGDIEDYYVGVASDVEQQGADAFDPEEYQFTCL
TYKESEGALNEHMTSLASVLKVSHSVAKLILVNFH
WQVSEILDRYKSNSAQLLVEARVQPNPSKHVPTSHP
PHHCAVCMQFVRKENLLSLACQHQFCRSCWEQHCSV
LVKDGVGVGVSCMAQDCPLRTPEDFVFPLLPNEEL
REKYRRYLFRDYVESHYQLQLCPGADCPMVIRVQE
PRARRVQCNRCNEVFCFKCRQMYHAPTDCATIRK
WLTKCADDSETANYISAHTKDCPKCNICIEKNG
GCNHMQCSKCKHDFCWMCLGDWKTHGSEYYECS
RYKENPDIVNQSQQAQAREALKKYLFYFERWENHNK
SLQLEAQTYQRIHEKIQERVMNNLGTWIDWQYLQ
NAAKLLAKCRYTLQYTYPYAYYMESGPRKKLFEY
QQAQLEAEIENLSWKVERADSYDRGDLENQMHI
AEQRRRTLLKDFHDT

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

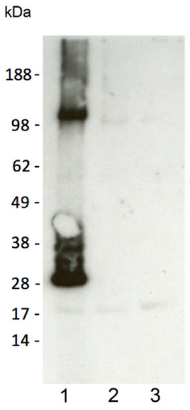
Quality Assurance

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.



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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: v1.0.1