

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and **Ubiguitylation Unit (University of** Dundee, Dundee, UK).

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 ubiguitylation; 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 elF4E2 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).

Antibody Production:

Anti-TRIAD1 (human) polyclonal antibody was raised in sheep against TRIAD1 (residues 1-493 of human TRIAD1). The antibodies were

Continued on page 2



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TRIAD1 (human; full length), pAb

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

Cat.	No.	e	
Lot.	No.	3	3

8-0023-100 0260

Quantity: Storage:

Page 1 of 2

100 µg -20°C

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CERTIFICATE OF ANALYSIS

NOT FOR USE IN HUMANS

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human TRIAD1 (residues 1 - 493

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects TRIAD1 at ~58 kDa

Reactivity: human, chicken and mouse

Stability/Storage: 12 months at -20°C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting: Use 0.1-0.5 µg/ml

Immunoprecipitation: Use 2.0 µg/mg of cell extract



Western Blotting Analysis:

U2OS cells were transfected with control siRNA, TRIAD1-1 siRNA, TRIAD1-2 siRNA or TRIAD1-3 siRNA (lanes 1, 2, 3 and 4 respectively). By Western blotting the specific recognition of a band corresponding to TRIAD1 was observed in lysates treated with control siRNA (lane 1) compared to lysates treated with TRIAD1-1-3 siRNAs where the presence of TRIAD1 could either not be detected (lane 2) or was significantly reduced (lanes 3 and 4) when probed with 0.5 µg/ml anti-TRIAD1 antibody (Cat# 68-0023-100).

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Background

Continued from page 1

purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-TRIAD1 pAbs from the sheep serum using a GSTtagged antigen-agarose column. Anti-TRIAD1 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General 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.

Application Reference:

Kelsall IR, Duda DM, Olszewski JL, Hofmann K, Knebel A, Langevin F, Wood N, Wrightman M, Schulman BA, and Alpi AF (2013) TRIAD1 and HHARI bind to and are activated by distinct neddylated Cullin-RING ligase complexes. *EMBO J* **32**, 2848-60.

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