

TRIAD1 (human; full length), pAb

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

Cat. No. 68-0023-100
Lot. No. 30260

Quantity: 100 µg
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

Page 1 of 2

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation 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 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) (Martijn *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).

Antibody Production:

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

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

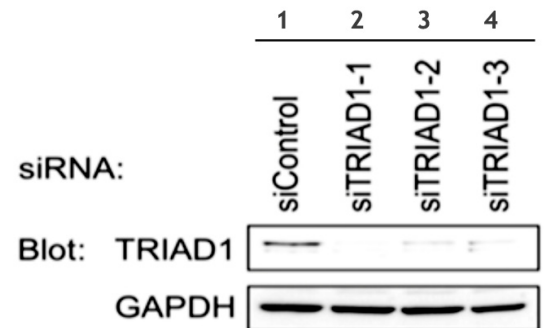
Stability/Storage: 12 months at -20°C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.1-0.5 µg/ml

Immunoprecipitation:
Use 2.0 µg/mg of cell extract

RNA interference experiment in U2OS cells showing specificity of antibody:



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).

Continued on page 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

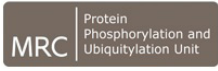
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0



TRIAD1 (human; full length), pAb

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

Cat. No. 68-0023-100
Lot. No. 30260

Quantity: 100 µg
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

Page 2 of 2

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 GST-tagged antigen-agarose column. Anti-TRIAD1 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Marteijn JA, van Emst L, Erpelinc-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, Erpelinc-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) UBC7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature* **474**, 105-8.

Application Reference:

Kellsall 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.



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.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0