





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

Background

ABIN1 contains a polyubiquitin-binding domain homologous to that present in nuclear factor κB (NF-κB) essential modulator (NEMO), a component of the inhibitor of NF-κB (IκB) kinase (IKK) complex (Nanda et al., 2011). The interaction of ABIN1 with polyubiquitin is required to limit the activation of the TLR (Toll-like receptor) signalling pathway and prevent autoimmunity (Nanda et al., 2011). ABIN1 has been implicated in autoimmune disorders such as systemic lupus erythematosus (SLE), diabetes mellitus and psoriasis (Gao et al., 2011; Zhou et al., 2011) and has the ability to inhibit TNF-induced apoptosis (Verstrepena et al., 2009). The TLR pathway activates diverse signal-transduction pathways that control proinflammatory effector mechanisms, such as production of cytokines, growth factors and cytotoxic mediators (Zhou et al., 2011).

Antibody Production:

Anti-ABIN1 (human) polyclonal antibody was raised in sheep against ABIN1 (residues 1-636 of human ABIN1). The antibodies were 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-ABIN1 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-ABIN1 (hu-

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ABIN1 (human; full length), pAb

Alternate Names: A20-binding inhibitor of NF-kappa-B activation 1, HIV-1 Nef-interacting protein, Nef-associated factor 1, Nip40-1, Virion-associated nuclear shuttling protein, TNFAIP3-interacting protein 1

Cat. No. 68-0002-100 Quantity: 100 µg Lot. No. -20°C 30239 Storage:

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on

shipping

Source: sheep polyclonal antibody

Immunogen: human ABIN1 (residues

1-636) [GST-tagged]

Purification: affinity-purified using

immobilized immunogen

Formulation: phosphate-buffered

Specificity: detects ABIN1 at ~72 kDa

Reactivity: human; other species not

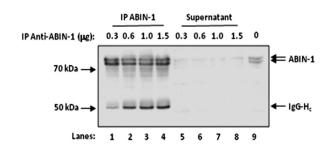
tested

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

Research Applications and Quality Assurance

Western Immunoblotting: Use 0.5 µg/ml

Immunoprecipitation: Use 1 µg/mg of cell extract



Immunoprecipitation Assay:

HEK293 cells stably expressing IL-1R were serum starved for 16h. ABIN1 was immunoprecipitated from total cell extracts (1 mg) using various amounts of anti-ABIN1 antibody (Cat# 68-0002-100). ABIN1 was subsequently detected by Western Blot using the anti-ABIN1 antibody (Cat# 68-0002-100). In order to show that all ABIN1 was immunoprecipitated from the input cell extract, a Western Blot was carried out using anti-ABIN1 antibody (Cat# 68-0002-100) on 25 µg of the cell extract following immunoprecipitation - 'Supernatant' - and no ABIN1 could be detected. This demonstrates that 1 µg of the ABIN1 antibody (Cat# 68-0002-100) can completely deplete ABIN1 from 1 mg of cell extracts. N.B: ABIN1 runs as a doublet just above the 70 kDa marker.

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Lot-specific COA version tracker: v1.0.0







Background

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man) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Gao L, Coope H, Grant S, Ma A, Ley S and Harhaj E (2011) ABIN1 Protein Cooperates with TAX1BP1 and A20 Proteins to Inhibit Antiviral Signaling. J Biol Chem 286, 36592–36602.

Nanda S, Venigalla R, Ordureau A, Patterson-Kane J, Powell D, Toth R, Arthur J, and Cohen P (2011) Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. J Exp Med 208, 1215-1228.

Verstrepena L, Carpentiera I, Verhelsta K and Beyaert R (2009) ABINs: A20 binding inhibitors of NF-κB and apoptosis signaling. *Biochem Phar*macol 78, 105-114.

Zhou J, Wu R, High A, Slaughter C, Finkelstein D, Rehg J, Redecke V and Häckera H (2011) A20-binding inhibitor of NF- κ B (ABIN1) controls Toll-like receptor-mediated CCAAT/enhancer-binding protein β activation and protects from inflammatory disease. Proc Natl Acad Sci USA 108, 998-1006.

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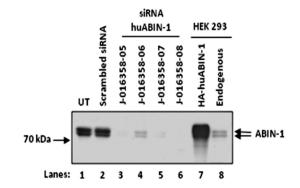
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CERTIFICATE OF ANALYSIS Page 2 of 2

Research Applications and Quality Assurance

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Western Blotting Analysis:

HeLa cells were transfected for 48 h with either scrambled siRNA (lane 2) or four different human ABIN1 siRNA (lanes 3-6). HEK293 cells were transfected for 24 h with DNA encoding HA-ABIN1 (lane 7). Lanes 1 and 8 are untreated HeLa and HEK293 cells, respectively. The cells were then lysed and the lysates denatured in SDS and subjected to SDS-PAGE on 8% gels. Western blotting was carried out with 0.5 μg/ml anti-ABIN1 (Cat# 68-0002-100).



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