

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
Lot. No. 30239

Quantity: 100 µg
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

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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 (Verstrepen *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-

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 saline

Specificity: detects ABIN1 at ~72 kDa

Reactivity: human; other species not tested

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

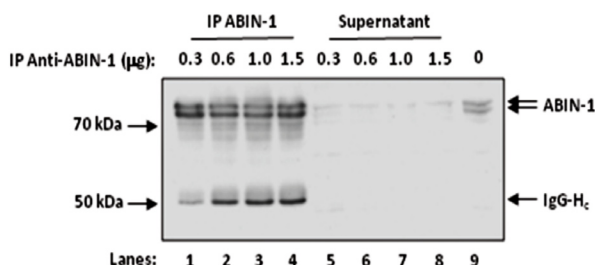
Research Applications and Quality Assurance

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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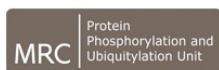
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Lot-specific COA version tracker: v1.0.0



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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.

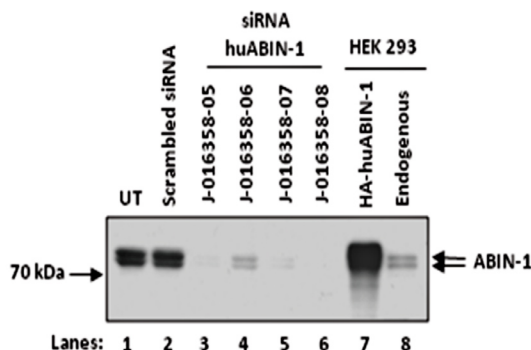
Nanda S, Venigalla R, Ordureau A, Patterson-Kane J, Powell D, Toth R, Arthur J, and Cohen P (2011) Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. *J Exp Med* **208**, 1215–1228.

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

Zhou J, Wu R, High A, Slaughter C, Finkelstein D, Reh J, Redecke V and Häcker H (2011) A20-binding inhibitor of NF-κB (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.

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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