SHARPIN (human; full length), pAb

Alternate Names: Shank-associated RH domain-interacting protein, Shank-interacting protein-like 1

Cat. No.	**68-0021-100**	Quantity:	100 µg
Lot. No.	**30258**	Storage:	-20˚C

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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 linear ubiquitin chain assembly complex (LUBAC) mediates linear polyubiquitination of proteins (Verhelst *et al.*, 2012) through ubiquitylation of the amino-terminal methionine of ubiquitin, repeated linear chain extension and attachment of such chains to the target substrate (Reiser E *et al.*, 2012). It is an E3 ubiquitin ligase complex composed of a catalytic subunit HOIP (HOIL-1-interacting protein) and the two regulatory subunits HOIL-1 (haem-oxidized iron-regulatory protein 2 ubiquitin ligase-1) and SHARPIN (SHANK-associated RH domain-interacting protein) (Verhelst *et al.*, 2012; Tokunaga & Iwai 2012). LUBAC plays an important role in TNF-induced NF-kB signalling (Haas *et al.*, 2009; Tokunaga *et al.*, 2009) and is involved in inflammatory responses, acquired and innate immunity, lymphocyte development, interferon production, the genotoxic stress response, and skeletal conditions. LUBAC has been implicated in various inflammatory, infectious and autoimmune diseases such as psoriasis-like dermatitis, rheumatoid arthritis, sepsis, and systemic lupus erythematosus (Tokunaga & Iwai, 2012). Various tumour tissues show enhanced SHARPIN expression which suggest a role for LUBAC in carcinogensis (Jung *et al.*, 2010).

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human SHARPIN (residues 1-326) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects SHARPIN at ~40 kDa

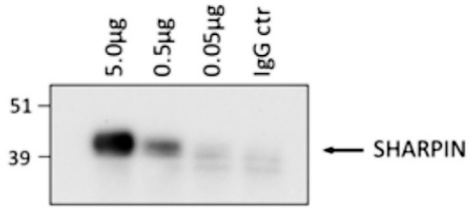
Reactivity: human; other species not tested

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

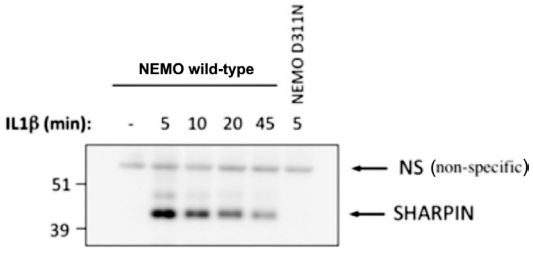
Research Applications and Quality Assurance

Western Immunoblotting:
Use 1 µg/ml

Immunoprecipitation:
Use 5 µg/mg of cell extract



Immunoprecipitation Assay:
SHARPIN was immunoprecipitated from HeLa total cell extracts (1 mg) using various amounts of SHARPIN antibody (Cat# 68-0021-100). SHARPIN was subsequently detected by Western Blot using a commercially available anti-SHARPIN antibody.



Western Blotting Analysis:
HEK293 IL-1R cells were incubated with or without IL-Iβ, SHARPIN was immunoprecipitated from 3 mg cell lysates using immobilised NEMO (IKKγ), NEMO (Cat# 66-1002-050) captures linear and K63-linked ubiquitin chains. Western Blotting was carried out on eluted proteins using anti-SHARPIN antibody (Cat# 68-0021-100). The results show that NEMO captures SHARPIN only in IL1-stimulated cells. SHARPIN was not captured, when NEMO was replaced by the polyubiquitin-binding defective mutants NEMO D311N (Cat# 66-1013-050).

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

Lot-specific COA version tracker: v1.0.0

SHARPIN (human; full length), pAb

Alternate Names: Shank-associated RH domain-interacting protein, Shank-interacting protein-like 1

Cat. No.	**68-0021-100**	Quantity:	100 µg
Lot. No.	**30258**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

Antibody Production:
Anti-SHARPIN (human) polyclonal antibody was raised in sheep against SHARPIN (residues 1-326 of human SHARPIN). 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-SHARPIN pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-SHARPIN (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Haas TL, Emmerich CH, Gerlach B, Schmukle AC, Cordier SM *et al.*, (2009) Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signalling complex and is required for TNF-mediated gene induction. *Mol Cell* **36**, 831–844.

Jung J, Kim JM, Park B, Cheon Y, Lee B, *et al.*, (2010) Newly identified tumor-associated role of human Sharpin. *Mol Cell Biochem* **340**, 161–167.

Rieser E, Cordier SM, Walczak H (2013) Linear ubiquitination: a newly discovered regulator of cell signalling. *Trends in Biochemical Sciences* **38**, 94-102.

Tokunaga F, Iwai K (2012) LUBAC, a novel ubiquitin ligase for linear ubiquitination, is crucial for inflammation and immune responses. *Microbes and Infection* **14**, 563–572.

Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T *et al.*, (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* **11**, 123–132.

Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensche T, Dikic I, Beyaert R (2012) A20 inhibits LUBAC-mediated NF-κB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* **31**, 3845–3855.

Application Reference:

Emmerich CH, Ordureau A, Strickson S, Arthur JSC, Pedriolo PGA, Komander D and Cohen P (2013) Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *PNAS* **110**, 15247-52.

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