HOIL1 (human; full length), pAb

Alternate Names: Heme-oxidized IRP2 ubiquitin ligase 1, RanBP-type and C3HC4-type zinc finger-containing protein 1, HBV-associated factor 4, Hepatitis B virus X-associated protein 4, RING finger protein 54, Ubiquitin-conjugating enzyme 7-interacting protein 3

Cat. No.	**68-0012-100**	Quantity:	100 µg
Lot. No.	**30249**	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 polyubiquitylation 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 *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 (heme-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 suggests a role for LUBAC in carcinogenesis (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 HOIL1 (residues 1-510) [His-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects HOIL1 at ~58 kDa

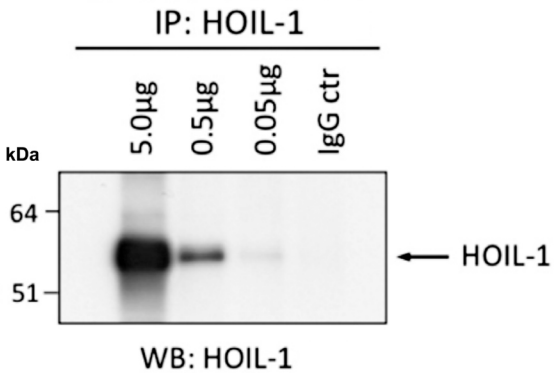
Reactivity: human; other species not tested

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:
HOIL1 was immunoprecipitated from HeLa total cell extracts (1 mg) using various amounts of anti-HOIL1 antibody (Cat# 68-0012-100) or pre-immune serum (IgG). HOIL1 was subsequently detected by Western Blot using a commercially available anti-HOIL1 antibody.

Continued on page 2

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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

HOIL1 (human; full length), pAb

Alternate Names: Heme-oxidized IRP2 ubiquitin ligase 1, RanBP-type and C3HC4-type zinc finger-containing protein 1, HBV-associated factor 4, Hepatitis B virus X-associated protein 4, RING finger protein 54, Ubiquitin-conjugating enzyme 7-interacting protein 3

Cat. No.	**68-0012-100**	Quantity:	100 µg
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CERTIFICATE OF ANALYSIS | **Page 2 of 2**

Background

Continued from page 1

Antibody Production:
Anti-HOIL1 (human) polyclonal antibody was raised in sheep against HOIL1 (residues 1-510 of human HOIL1). 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-HOIL1 pAbs from the sheep serum using a 6His-tagged-antigen-agarose column. Anti-HOIL1 (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 JM, Kim B, Park Y, Cheon 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.

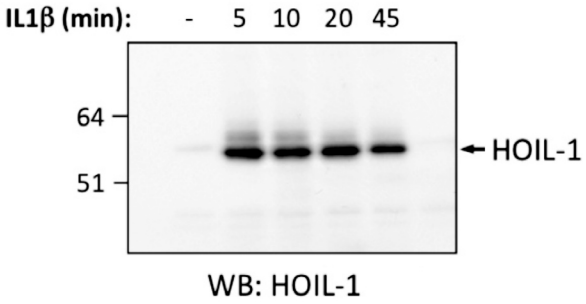
Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensche T, Dikic I, Beyaert R (2012) A20 inhibits LUBAC-mediated NF-κB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* **31**, 3845–3855.

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.

Research Applications and Quality Assurance

Continued from page 1



WB: HOIL-1

Western Blotting Analysis:
HEK293 IL-IR expressing cells were incubated with or without IL-Iβ for varying amounts of time, HOIL1 was precipitated 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-HOIL1 antibody (Cat# 68-0012-100). The results show that NEMO captures HOIL1 from IL1β-stimulated and not unstimulated cells. HOIL1 was not captured when NEMO was replaced by the polyubiquitin-binding defective mutant NEMO D311N (data not shown; See Cat# 66-1013-050).

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Lot-specific COA version tracker: v1.0.0