

## HOIP (human; full length), pAb

Alternate Names: RNF31, HOIL 1L interacting protein, ZIBRA, Zinc in between ring finger ubiquitin associated domain

Cat. No. 68-0013-100  
Lot. No. 30250

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 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 *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-κB 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 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 HOIP (residues 1-1072) [GST-tagged]

**Purification:** affinity-purified using immobilized immunogen

**Formulation:** phosphate-buffered saline

**Specificity:** detects HOIP at ~120 kDa

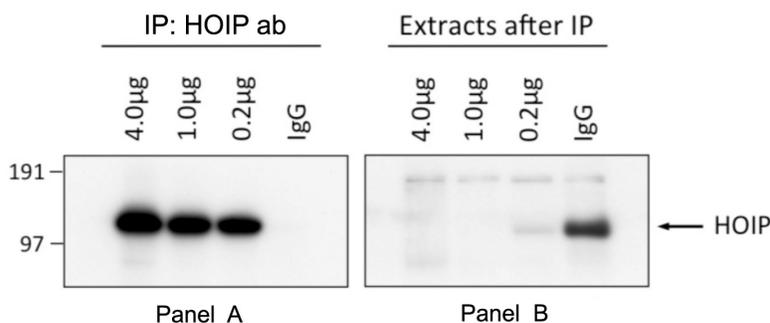
**Reactivity:** human; other species not tested

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

### Research Applications and Quality Assurance

**Western Immunoblotting:**  
Use 1 µg/ml

**Immunoprecipitation:**  
Use 1 µg/mg of cell extract



#### Immunoprecipitation Assay:

HOIP was immunoprecipitated from HeLa total cell extracts (1 mg) using various amounts of HOIP antibody (Cat# 68-0013-100) or pre-immune serum (IgG). HOIP was subsequently detected using a commercially available anti-HOIP antibody (Panel A). In order to show that all HOIP was immunoprecipitated from the input cell extract, a Western Blot was carried out using anti-HOIP antibody (Cat# 68-0013-100) on 20 µg of the cell supernatant following immunoprecipitation and no HOIP could be detected (Panel B). This demonstrates that 1 µg of the anti-HOIP antibody (Cat# 68-0013-100) can completely deplete HOIP from 1 mg of cell extracts.

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



## HOIP (human; full length), pAb

Alternate Names: RNF31, HOIL 1L interacting protein, ZIBRA, Zinc in between ring finger ubiquitin associated domain

Cat. No. 68-0013-100  
Lot. No. 30250

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

### Antibody Production:

Anti-HOIP (human) polyclonal antibody was raised in sheep against HOIP (residues 1-1072 of human HOIP). 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-HOIP pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-HOIP (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.

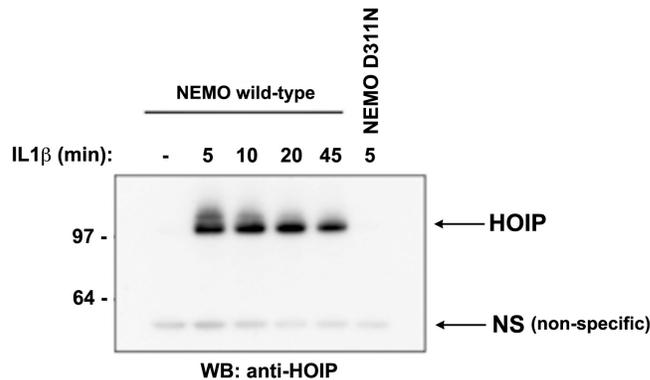
Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensche T, Dikic I, Beyaert R (2012) A20 inhibits LUBAC-mediated NF-κB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* 31, 3845–3855.

### Application Reference:

Emmerich CH, Ordureau A, Strickson S, Arthur JSC, Pedriolo PGA, Komander D, 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



### Western Blotting Analysis:

HEK293-IL1R expressing cells were incubated with or without IL-1β for varying amounts of time, HOIP 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-HOIP antibody (Cat# 68-0013-100). The results show that NEMO captures HOIP from IL1β-stimulated and not unstimulated cells. HOIP was not captured, when NEMO was replaced by the polyubiquitin-binding defective mutant NEMO D311N (Cat# 66-1013-050).



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