Optineurin (human; full length), pAb

Alternate Names: E3-14.7K-interacting protein, FIP-2, Huntingtin yeast partner L, Huntingtin-interacting protein 7, NEMO-related protein, Optic neuropathy-inducing protein

Cat. No.	**68-0015-100**	Quantity:	100 µg
Lot. No.	**30252**	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

Ubiquitin signals are decoded in cells by at least 200 ubiquitin binding proteins, which interact with different types of polyubiquitin chains and ubiquitin-like modifiers. These interactions induce conformational changes that allow these proteins to transmit the ubiquitin signal to effector proteins (Dikic *et al.*, 2009). Optineurin is a protein that is most closely related to NFκB Essential Modifier (NEMO) and, like NEMO, it contains a domain that binds to both Lys63-linked and linear polyubiquitin chains (Gleason *et al.*, 2011). These polyubiquitin chains can then regulate downstream signalling events by inducing conformational changes that activate protein kinases such as IκB kinase (IKK) or Tank binding kinase (TBK1) (Gleason *et al.*, 2011). TBK1 can also phosphorylate optineurin at Ser177, enhancing its interaction with the microtubule-associated protein light chain 3 (LC3) which in turn promotes the autophagic clearance of ubiquitylated cytosolic Salmonella (Wild *et al.*, 2011). Mutations in optineurin cause three different diseases in humans, namely a form of glaucoma (Rezaie *et al.*, 2002), Paget's disease of bone (Albagha *et al.*, 2010) and amyotrophic lateralsclerosis (ALS), a form of motor neurone disease (Maruyama, *et al.*, 2010).The Optineurin [E478G] mutation, which causes ALS, abolishes binding to polyubiquitin chains (Gleason *et al.*, 2011).

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human Optineurin (residues 1-557)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Optineurin at ~66 kDa

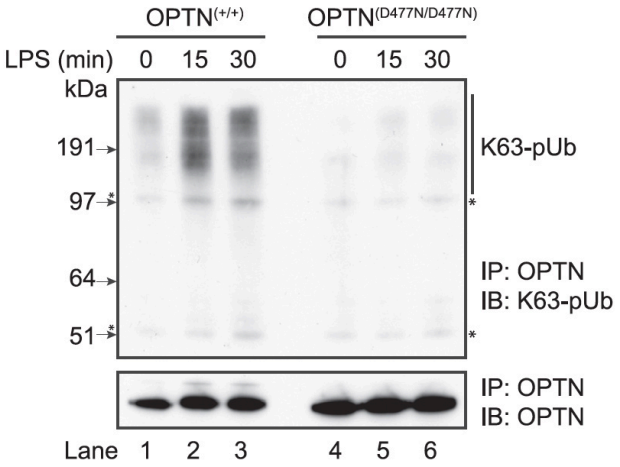
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
Not tested

Immunoprecipitation:
Use 4 µg/mg of cell extract



Immunoprecipitation Assay:
Optineurin was immunoprecipitated from bone marrow derived macrophages (10 mg) derived from Optineurin$^{+/+}$ or Optineurin$^{D477N/D477N}$ mice stimulated with lipopolysaccharide (LPS) (100 ng/ml) using 40 µg of Optineurin antibody (Cat# 68-0015-100). Optineurin's ability to act as a ubiquitin binding protein was observed by Western Blotting for K63-linked ubiquitin chains.

N.B: The asterisks denote nonspecific bands.

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Lot-specific COA version tracker: v1.0.0

Optineurin (human; full length), pAb

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CERTIFICATE OF ANALYSIS |

Background

Continued from page 1

Antibody Production:
Anti-Optineurin (human) polyclonal antibody was raised in sheep against Optineurin (residues 1-557 of human Optineurin). 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-Optineurin pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-Optineurin (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, *et al.* (2010) Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone. *Nature Genetics* **42**, 520524.

Dikic I, Wakatsuki S and Walters KJ (2009) Ubiquitin-binding domains - from structures to functions. *Nat Rev Mol Cell Biol* **10**, 659-671.

Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, *et al.* (2010) Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* **465**, 223-226.

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, *et al.* (2002) Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science* **295**, 1077-1079.

Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, *et al.* (2011) Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science* **333**, 228-233.

Application Reference:

Gleason CE, Ordureau A, Gourlay R, Arthur JS and Cohen P (2011) Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon beta. *J Biol Chem* **286**, 35663-35674.

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