

# Optineurin (D474N) [GST-tagged]

## Ubiquitin Binding Protein

Alternate Name: OPTN, NEMO related protein, Transcription factor III interacting protein

Cat. No. 66-1014-050

Lot. No. 30102

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

### 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 signaling 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 lateral sclerosis (ALS), a form of motor neuron disease (Maruyama *et al.*, 2010). The Optineurin [E478G] mutation, which causes ALS, abolishes binding to polyubiquitin chains (Gleason *et al.*, 2011). Optineurin is a powerful reagent for capturing the Lys63-linked

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### Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5,  
150 mM sodium chloride,  
2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~92.8 kDa

Purity: >70% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C;  
aliquot as required

Protein Sequence: Please see page 2

### Quality Assurance

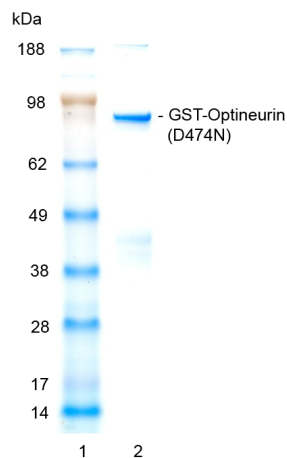
#### Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg GST-Optineurin-  
(D474N)

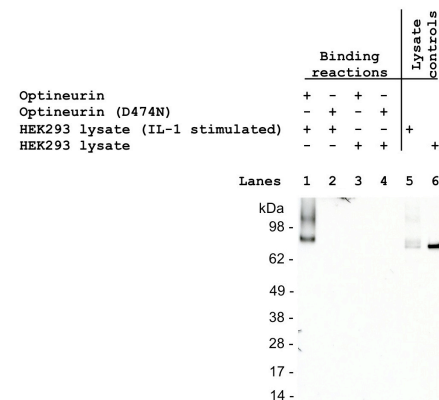


#### Protein Identification:

Confirmed by mass spectrometry.

#### Ubiquitin Binding Domain Activity:

The lack of GST-Optineurin (D474N) ubiquitin chain binding domain activity was determined through its inability to capture poly-ubiquitylated IRAK1 from a lysate preparation derived from IL-1 stimulated HEK293 cells. GST-Optineurin (D474N) was pre-incubated with Glutathione Sepharose 4B for 20 minutes at 4°C followed by an incubation for 2 hours at 4°C with 2mg IL-1 stimulated HEK293 cell lysate. The binding reaction was then centrifuged and the pellet analysed by SDS-PAGE/Western blotting (Lane 2). This sample was compared alongside similarly derived pull-downs from control reactions containing GST-Optineurin wild-type versus mutant (D474N) incubated in the presence of lysates derived from either IL-1 stimulated or non-stimulated HEK293 cells (Lanes 1-4). Ubiquitylated IRAK1 was identified by Western Blotting using an anti-IRAK1 antibody and such species were observed only in the pellet sample derived from a binding reaction containing wild-type GST-Optineurin and IL-1 stimulated HEK293 cell lysate (Lane 1).



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Lot-specific COA version tracker: v1.0.0

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### Background

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and linear polyubiquitin chains and their binding partners present in cell extracts. It is recommended that the Optineurin [D474N] mutant, which is unable to bind polyubiquitin chains, is used as a control in such experiments (Sudhakar *et al.*, 2009).

#### 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, 520-524.

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

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.

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.

Sudhakar C, Nagabhushana A, Jain N and Swarup G (2009) NF-kappaB mediates tumor necrosis factor alpha-induced expression of optineurin, a negative regulator of NF-kappaB. *PLoS One* 4, e5114.

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.

### Physical Characteristics

Continued from page 1

#### Protein Sequence:

**MSPILGYWKIKGLVQPTRLLLEYLEEKY**  
**EEHLYERDEGDKWRNKKFELGLEFPN**  
**LPYYIDGDVKLQSMAIIRYIADKHNLG**  
**GCPKERAEISMLEGAVLDIRYGVSRIAY**  
**SKDFETLKVDFLSKLPEMLKMFEDRLCHK**  
**TYLNGDHVTHPDFMLYDALDVVLYMDPM**  
**CLDAFPKLVCFKKRIEAIPOIDKYLKSSKY**  
**IAWPLQGWQATFGGGDHPKSDLEVLFGQ**  
**PLGSMHQPLSCLTEKEDSPSESTGNGP**  
**PHLAHPNLDTFTEELLQOMKELLTEN**  
**HOLKEAMKLNNQAMKGRFEELSAWTEKQKEER**  
**QFFEIQSKEAKERLMALSHENEKLKEELG**  
**KLKKGKSERSSEDPDSDSRLPRAEAEQEKDQL**  
**RTQVVRLQAEKADLLGIVSELQLKLN**  
**SGSSSEDSFVEIRMAEGEAEGSVKEIKHSPGP**  
**TRTVSTGTALSKYRSRSADGAKNYFEHEELT**  
**VSQLLCLREGNQKVERLEVALKEAKERSV**  
**DFEKTSNRSEIETQTEGSTEKENDEEKGPET**  
**VGSEVEALNLQVTSLFKELQEAHTKLSE**  
**AELMKRRLQEKQALERKNSAIPSEL**  
**NEKQELVYTNKKLELQVESMLSEIKMEQAK**  
**TEDEKSKLTVLQMTHNKLLQEHNNALK**  
**TIEELTRKESEKVDRAVLKELSEKLELAEKA**  
**LASKQLQMDMKQTIKQEEEDLETMTIL**  
**RAQMEVYCSNFHAERAAREKIHEEKEQLA**  
**LQLAVLLKENDAFEDGGROSLMEMQSRH**  
**GARTSDSDQQAYLVORGAEDRDWRQQRNIPIH**  
**SCPCKGEVLPDIDTLQIHVMDCII**

Tag (**bold text**): N-terminal GST  
Protease cleavage site: PreScission™ (LEVLFG▼GP)  
Optineurin (D474N) (regular text): Start **bold italics**  
(amino acid residues 1-577)  
Accession number: NP\_001008214



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