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 neurone 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

Continued on page 2

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

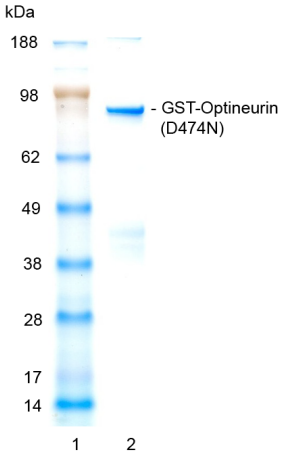
Purity: >70% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

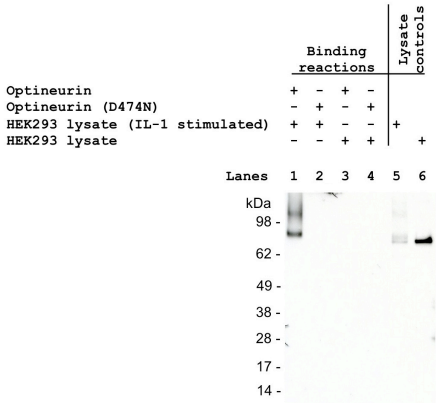
Quality Assurance

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 polyubiquitylated 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).



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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.

Lot-specific COA version tracker: v1.0.0

Optineurin (D474N) [GST-tagged]

Ubiquitin Binding Protein

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

Cat. No.	**66-1014-050**	Quantity:	50 µg
Lot. No.	**30102**	Storage:	-70˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS | **CERTIFICATE OF ANALYSIS Page 2 of 2**

Background

Continued from page 1

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
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGS***M***SHQPLSCLTEKEDSPSESTGNGP
PHLAHPNLDTFTPEELLQQMKELLTEN
HQLKEAMKLNNQAMKGRFEELSAWTEKQKEER
QFFEIQSKEAKERLMALSHENEKLKEELG
KLKGKSERSSEDPTDDSRLPRAEAEQEKDQL
RTQVVRLQAEKADLLGIVSELQLKLNS
SGSSEDSFVEIRMAEGEAEGSVKEIKHSPGP
TRTVSTGTALSKYRSRSADGAKNYFEHEELT
VSQLLLCLREGNQKVERLEVALKEAKERVS
DFEKKTSNRSEIETQTEGSTEKENDEEKGPET
VGSEVEALNLQVTSLFKELQEAHTKLSE
AELMKKRLQEKCQALERKNSAIPSEL
NEKQELVYTNKKLELQVESMLSEIKMEQAK
TEDEKSKLTVLQMTHNKLLQEHNNALK
TIEELTRKESEKVDRAVLKELSEKLELAEKA
LASKQLQMDEMKQTIAKQEEDLETMTIL
RAQMEVYCSNFHAERAAREKIHEEKEQLA
LQLAVLLKENDAFEDGGRQSLMEMQSRH
GARTSDSDQQAYLVQRGAEDRDWRQQRNIPIH
SCPKCGEVLPDIDTLQIHVMDCII

Tag **(bold text):** N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
Optineurin (D474N) (regular text): Start ***bold italics***
(amino acid residues 1-577)
Accession number: NP_001008214

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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.

Lot-specific COA version tracker: v1.0.0