PINK1 (D359A) [MBP-tagged]

Kinase

Alternate Name: PTEN-Induced Putative Kinase 1

Cat. No.	**66-0044-050**	Quantity:	50 µg
Lot. No.	**30343**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are two major post-translational modifications that regulate the functions of proteins in eukaryotic cells. However, these modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of PTEN Induced putative Kinase 1 (PINK1) was first described by Unoki and Nakamura *et al.* (2001). PINK1 is a mitochondrial serine/threonine kinase involved in the normal function and integrity of mitochondria, PINK1 reduces neuronal apoptosis through a reduction in cytochrome c release from mitochondria and subsequent activation of caspase 3 (Petit *et al.*, 2005). PINK1 has been shown to phosphorylate Parkin at Ser65 - located in its Ubl domain - which leads to a marked activation in the activity of the E3 ligase (Kondapalli *et al.*, 2012). PINK1 activation of Parkin catalyses K63-linked polyubiquitylation and enhances parkin-mediated ubiquitin signalling through the I-kappa-B kinase/nuclear factor kappa-B (NF-kappa-B) pathway. It is thought that deregulation of this pathway through Parkinson's Disease (PD)-linked mutations in PINK1 is the cause of PD pathogenesis (Sha *et al.*, 2010). PINK1

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Physical Characteristics

Species: Tribolium castaneum

Source: *E. coli*

Quantity: 50 µg

Concentration: 2.49 mg/ml

Formulation: 50 mM Hepes pH 7.5, 150 mM NaCl, 2 mM DTT, 10% glycerol

Molecular Weight: ~108.1 kDa

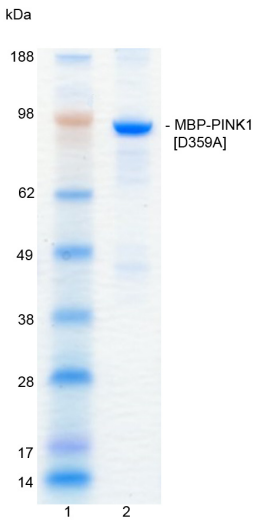
Purity: >85% by InstantBlue™ SDS-PAGE

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

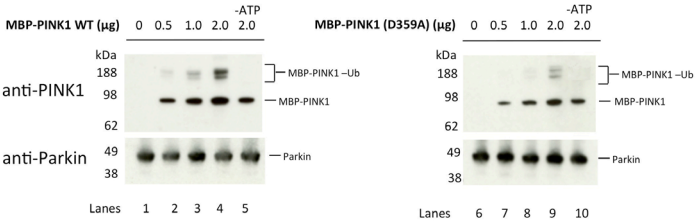
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg MBP-PINK1 [D359A]



Protein Identification:
Confirmed by mass spectrometry.



Kinase activity assay:
The kinase dead MBP-PINK1 (D359A) mutant was run as a control in parallel with MBP-PINK1 when assessing the ability of MBP-PINK1 to phosphorylate and activate Parkin enabling Parkin-catalysed generation of MBP-PINK1 ubiquitin conjugates. MBP-PINK1 (0, 0.5, 1.0 and 2.0 µg) or MBP-PINK1 (D359A) (0, 0.5, 1.0 and 2.0 µg) were incubated in kinase assay buffer with Parkin (2.0 µg) in the presence or absence of ATP for 60 minutes at 30˚C. The ubiquitylation reactions were then initiated through the addition His-UBE1, the E2 conjugating enzyme His-UBE2L3 (UbcH7) and ubiquitin and incubated for a further 60 minutes at 30˚C. MBP-PINK1 ubiquitin conjugates were identified by Western blotting using an anti-PINK1 antibody (lanes 3, 4 and 9) these were observed predominantly in the presence of ATP and WT PINK1 over those observed in the presence of ATP and the kinase dead MBP-PINK1 (D359A) mutant (compare lanes 4 and 9 respectively). Ubiquitin conjugates were not observed in the absence of MBP-PINK1 (lanes 1 and 6) or ATP (lanes 5 and 10).

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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

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Background

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controls Parkin E3 ligase activity not only by phosphorylating Parkin, but also by phosphorylating ubiquitin – both at Ser65. It is thought that phosphorylation of Parkin serves to prime the E3 ligase enzyme for activation by ubiquitin (pSer65) (Kazlauskaite *et al*. 2014). USP30 (a deubiquitylase (DUB) localized to mitochondria) antagonizes mitophagy driven by Parkin and PINK1. Parkin ubiquitylates and tags damaged mitochondria for clearance. USP30 removes ubiquitin attached by Parkin onto damaged mitochondria and blocks Parkin's ability to drive mitophagy. Thus USP30 inhibition is potentially beneficial in Parkinson's disease by promoting mitochondrial clearance and quality control (Bingol *et al*. 2014).

References:

Bingol B, Tea JS, Phu L, Reichelt M, Bakalarski CE, Song Q *et al.* (2014) The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* **510**, 370-5.

Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, Hofmann K et al. (2014) Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J* **460**, 127-139.

Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R, Burchell L, Walden H, MacCartney TJ, Deak M, Knebel A, Alessi DR and Muqit MM (2012) PINK1 is activated by mitochondrial membrane potential depolarization and stimulates PARKIN E3 ligase activity by phosphorylating Serine 65. *Open Biology* **5**, 120080.

Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, Chen F, Gu Y, Hasegawa H, Salehi-Rad S, Wang L, Rogaeva E, Fraser P, Robinson B, St George-Hyslop P, Tandon A (2005) Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* **280**, 34025-34032.

Sha D, Chin LS, Li L (2010) Phosphorylation of parkin by Parkinson disease-linked kinase PINK1 activates parkin E3 ligase function and NF-kappa-B signaling. *Hum Molec Genet* **19**, 352-363.

Unoki M, Nakamura, Y (2001) Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. *Oncogene* **20**, 4457-4465.

Physical Characteristics

Continued from page 1

Protein Sequence:

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDT
GIKVTVEHPDKLEEKFPQVAATGDGPDIIF
WAHDRFGGYAQSGLLAEITPDKAFQDKLYP
FTWDAVRYNGKLIAYPIAVEALSLIYNKDLLP
NPPKTWEEIPALDKELKAKGKSALMFNLQEPY
FTWPLIAADGGYAFKYENGKYDIKDVGVDNA
GAKAGLTFLVDLIKNKHMNADTDYSIAEAAF
NKGETAMTINGPWAWSNIDTSKVNYGVTVLPT
FKGQPSKPFVGVLSAGINAASPNKELAKE
FLENYLLTDEGLEAVNKDKPLGAVALKSY
EEELVKDPRIAATMENAQKGEIMPNIPQMSAF
WYAVRTAVINAASGRQTVDEALKDAQTNS
SSNNNNNNNNNNLGDDDDKVPEFLEVLFQG
PGS***M***SVRAVGSRLFKHGRSLIQQFCKRDLNT
TIGDKINAVSQATAAPSSLPKTQIPKNFAL
RNVGVQLGLQARRILIDNVLNRVTNSL
SAELRKKATRRILFGDSAPFFALVGVSIAS
GTGILTKEEELEGVCWEIREAISKIKWQYY
DIDESRFESNPITLNDLSLGKPIAKGTNGV
VYSAKVKDDETDDNKYPFALKMMFNYDIQSNS
MEILKAMYRETVPARMYYSNHDLNNWEIELAN
RRKHLPPHPNIVAIFSVFTDLIQELEGSKD
LYPAALPPRLHPEGEGRNMSLFLLMKRYDCN
LQSFLSTAPSTRTSLLLLAQLLEGVAHMTAH
GIAHRDLKSDNLLLDTSEPESPILVIS**A**FGC
CLADKTNGLSLPYTSYEMDKGGNTALMAPEI
ICQKPGTFSVLNYSKADLWAVGAIAYEIF
NCHNPFYGPSRLKNFNYKEGDLPKLPDEVPT
VIQALVANLLKRNPNKRLDPEVAANVCQLFL
WAPSTWLKPGLKVPTSGEILQWLLSLTTKVL
CEGKINNKSFGEKFTRNWRRTYPEYLLISSFL
CRAKLANVRNALHWIQENLPELD

Tag (**bold text**): N-terminal MBP
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
PINK1 (regular text): Start ***bold italics*** (amino acid residues 1-570)
Accession number: XP_968367.1

MBP-PINK1 enzyme carries a D359A mutation which yields a 'kinase dead' enzyme. Residue A359 (highlighted in red) of the kinase dead enzyme sequence is equivalent to D359 of the native wildtype sequence.

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