PINK1 [MBP-tagged]

Kinase

Alternate Name: PTEN-Induced Putative Kinase 1

Cat. No. **66-0043-050**
Lot. No. **30423**

Quantity: 50 µg
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 patho-

Continued on page 2

Physical Characteristics

Species: Tribolium castaneum

Source: *E. coli*

Quantity: 50µg

Concentration: 2.8 mg/ml

Formulation: 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCL, 0.1% ß-Mercaptoethanol, 270mM Sucrose, 0.03% Brij-35, 1mM Benzamidine, 0.2mM PMSF.

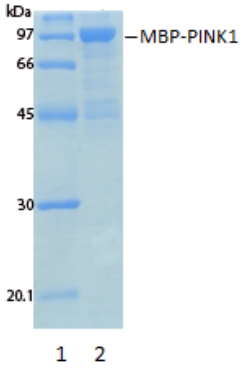
Molecular Weight: ~108.1 kDa

Purity: >85% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Purity:
12% SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5µg MBP-PINK1



Protein Identification:
Confirmed by mass spectrometry.

Activity assay:
MBP-PINK1 (Tribolium) (diluted in 50 mM Tris/HCl pH 7.5, 10 mM DTT, 0.1mM EGTA, 1 mg/ml BSA) was assayed in 50µl of 50 mM Tris/HCl pH 7.5, 10 mM DTT, 0.1 mM EGTA, GST-PARK2, 10 mM magnesium acetate, 100µM [γ- ^{32}P]-ATP (50-1000 cpm/pmole), at 30°C for 10 mins. Reactions were stopped by spotting 40µl out of the 50µl assay mixture onto 1.5cm x 1.5cm squares of Whatman P81 paper, which were washed in 75mM phosphoric acid, followed by acetone before air drying and counting.

MBP-PINK1 specific activity:
122.13 Units/mg (341.98 Units/ml)

1 unit = 1nmole of phosphate incorporated into the substrate in 1 minute.

Substrate: GST-PARK2

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

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

genesis (Sha *et al.*, 2010). PINK1 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
GIAHRDLKSDNLLLDTSEPESPILVISDFGC
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

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UK HQ and TECHNICAL SUPPORT
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