PINK1 (human; residues 175 - 250), pAb

Alternate Names: PTEN Induced putative Kinase 1, Park6

Cat. No.	**68-0019-100**	Quantity:	100 µg
Lot. No.	**30256**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS |

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human PINK1 (residues 175-250) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects PINK1 at ~63 kDa

Reactivity: human

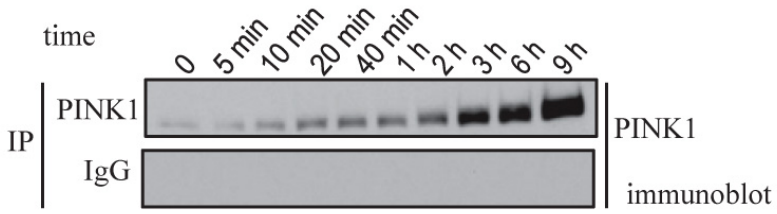
Species cross reactivity: mouse

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

Research Applications and Quality Assurance

Western Immunoblotting:
Use 1.0 µg/ml

Immunoprecipitation:
Use 5.0 µg/mg of cell extract



Immunoprecipitation Assay:
HEK293 cells were stimulated at the indicated time points with 10 mM of CCCP. HEK293 whole-cell lysates (1 mg) were immunoprecipitated with anti-PINK1 antibody (Cat# 68-0019-100) or pre-immune IgG covalently coupled to protein G Sepharose and resolved by 8% SDS-PAGE. Immunoblotting was performed with a commercially available anti-PINK1 (full length) antibody.

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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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Continued from page 1

deregulation of this pathway through Parkinson's Disease (PD)-linked mutations in PINK1 is the cause of PD pathogenesis (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).

Antibody Production:
Anti-PINK1 (human) polyclonal antibody was raised in sheep against PINK1 (residues 175-250 of human PINK1). 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-PINK1 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-PINK1 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General 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.

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.

Application Reference:

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.

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