# PINK1 pThr257 (human; residues 250-262), pAb

**Alternate Names:** PTEN Induced putative Kinase 1, Park6

| | | | |
|---|---|---|---|
| **Cat. No.** | **68-0057-100** | Quantity: | 100 µg |
| **Lot. No.** | **30358** | Storage: | -20˚C |

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

**CERTIFICATE OF ANALYSIS** | **Page 1 of 2**

**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 (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 is specifically activated by inducers of mitochondrial membrane depolarization (e.g. Carbonyl cyanide m-chlorophenyl hydrazone; CCCP) and once activated PINK1 autophosphorylates at several residues. The autophosphorylation of Thr257 on PINK1 serves as a reporter for PINK1 activation (Kondapalli *et al.*, 2012).

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## Physical Characteristics

**Quantity:** 100 µg

**Concentration:** to be provided on shipping

**Source:** sheep polyclonal antibody

**Immunogen:** human PINK1 (residues 250-262) [CAGEYGAV(pT)YRKSKR]

**Purification:** affinity-purified using immobilized immunogen

**Formulation:** phosphate-buffered saline

**Specificity:** detects PINK1 at ~63 kDa

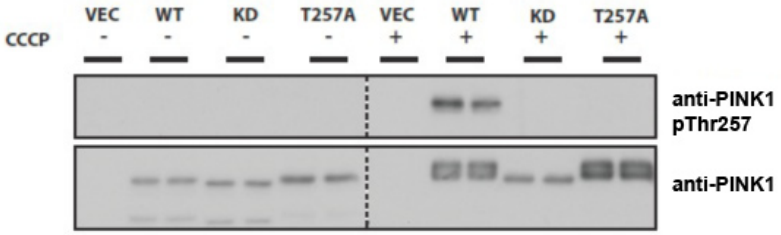
**Reactivity:** human; other species not tested

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

## Research Applications and Quality Assurance

**Western Immunoblotting:** use at 1 µg/ml; add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1011-001 provided) to your immunoblotting incubation per 1 µg of polyclonal antibody in order to deplete any non-phospho specific polyclonal antibodies present.

**Immunoprecipitation:** not tested




**Western Blotting Analysis:**
Flp In HEK293 stable cell lines expressing either FLAG alone (VEC), FLAG-PINK1 Wildtype (WT), FLAG-PINK1 Kinase dead (KD) or phospho dead mutant FLAG-PINK1 (T257A), were induced for expression with doxycyclin for 24 hrs. The cells were stimulated with either DMSO or CCCP (mitochondrial depolarizing agent) for 3 hrs and subjected to mitochondrial fractionation. 0.5 mg of mitochondrial extracts were immunoprecipitated with 5 µl of anti-FLAG agarose beads to immunoprecipitate recombinant PINK1. Proteins were resolved by 8% SDS-PAGE and subjected to western blot analysis probing with the anti-PINK1 pThr257 antibody (Cat# 68-0057-100) and the anti-PINK1 (full-length) antibody (Cat# 68-0019-100).

The antibody recognises mitochondrial Wild-type PINK1 only upon CCCP stimulation. It does not recognise PINK1 KD. Moreover, the specificity of the antibody is confirmed by loss of recognition of the phospho dead T257A PINK1 mutant.

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**Lot-specific COA version tracker:** v1.0.1

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**CERTIFICATE OF ANALYSIS** 

## Background

*Continued from page 1*

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 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 pThr257 (human) polyclonal antibody was raised in sheep against PINK1 pThr257 (residues 250-262 of human PINK1 ; Thr257 phosphorylated). 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 a GST-tagged antigen-agarose column. Anti-PINK1 pThr257 (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.

Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R, *et al.* (2012) PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol* **2**, 120080.

Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, *et al.* (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 and Li L (2010) Phosphorylation of parkin by Parkinson disease-linked kinase PINK1 activates parkin E3 ligase function and NF-kappaB signaling. *Hum Mol Genet* **19**, 352-363.

Unoki M and 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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