Parkin pSer65 (human; residues 60-72), pAb

Alternate Names: EC6.3.2, PRKN, AR-JP, Parkinson's disease protein 2

Cat. No.	**68-0056-100**	Quantity:	100 µg
Lot. No.	**30357**	Storage:	-20˚C

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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

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Parkin is a member of the E3 protein ligase family and cloning of the gene was first described by Asakawa *et al.* (2001). Mutations in Parkin cause autosomal recessive juvenile parkinsonism (AR-JP) that is distinct from sporadic PD by the general absence of cytoplasmic inclusions known as Lewy bodies (LBs). Parkinson's disease (PD) is characterized by the loss of dopamine neurons in the substantia nigra and the presence LBs (Muqit *et al.*, 2004). The failure of neurons to remove the misfolded proteins present in LBs and the identification of a mutation in Parkin provides evidence for the dysfunction of the ubiquitylation pathway in the disease (Muqit *et al.*, 2004; Shimura *et al.*, 2000). Studies have also identified the presence of at least five phosphorylation sites in Parkin including Ser378, shown to be phosphorylated by Casein kinase1 (CK1) and suggest that phosphorylation of Parkin may act to regulate its ubiquitin ligase activity (Yamamoto *et al.*, 2005). Parkin binds Ube2L6 through its c-

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human parkin (residues 60-72) [RDLDQQ(pS)IVHIVQR]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Parkin pSer65 at ~52 kDa

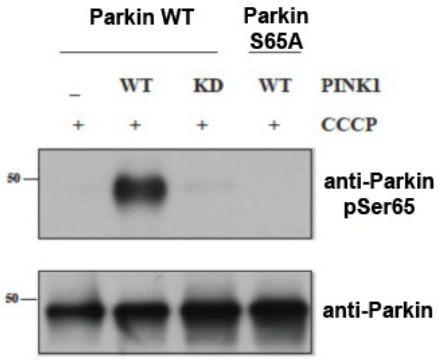
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
1 µg/ml; add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1010-001 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:
Untagged Parkin Wildtype (Parkin WT) was transiently transfected into Flp In HEK293 stable cell lines expressing either FLAG-tag alone (-), FLAG-PINK1 Wildtype (WT) or FLAG-PINK1- Kinase dead (KD). Parkin S65A mutant was transfected into FLAG-PINK1 WT stable cell lines. Cells were induced for PINK1 expression with doxycyclin for 24 hrs and stimulated with CCCP (mitochondrial depolarizing agent) for 3hrs. 0.25 mg of whole cell lysate was immunoprecipitated for Parkin with 5ul of Parkin antibody (Cat# 68-0018-100) coupled to Protein G sepharose. Proteins were resolved by SDS-PAGE and subjected to western blot analysis. The blot was probed with anti-Parkin pSer65 antibody (Cat# 68-0056-100) overnight at 4°C.

Anti-Parkin pSer65 antibody (Cat# 68-0056-100) recognises Parkin pSer65 when it is expressed in cells containing active PINK1 and not in PINK1 KD background. Also the specificity of the antibody is confirmed with loss of recognition of the Parkin S65A mutant.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.1

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Background

Continued from page 1

terminal domain and has been shown to ubiquitylate and degrade itself (Zhang *et al.*, 2000). Parkin Associated Endothelian Receptor Like Receptor (PAELR) is an insoluble protein that accumulates in the brains of Parkinson's Disease Juvenile (PDJ) patients, PAELR is a substrate of Parkin which specifically ubiquitylates and degrades insoluble PAELR in neurons (Imai *et al.*, 2001). In human neuroblastoma cells stressed by dopamine, proteasome inhibition, and proapoptotic stimuli, Parkin has been identified in aggresomes, co-localised with ubiquitin, however this has been shown to be variable, depending on the stress (Muqit *et al.*, 2004). PTEN Induced putative Kinase 1 (PINK1) has been shown to phosphorylate Parkin at Ser65 located in its Ubl domain which leads to a marked activation in the E3 ligase activity of Parkin. It is thought small molecule activators that mimic the effect of PINK1-catalysed phosphorylation could provide therapeutic benefit for PD sufferers (Kondapalli *et al.*, 2012). 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-Parkin pSer65 (human) polyclonal antibody was raised in sheep against Parkin pSer65 (residues 60-72 of human Parkin; Ser65 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-Parkin pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-Parkin pSer65 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Asakawa S, Tsunematsu K, Takayanagi A, Sasaki T, Shimizu A, Shintani A, *et al.* (2001) The genomic structure and promoter region of the human parkin gene. *Biochem Biophys Res Commun* **286**, 863-868.

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

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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.

Muqit MM, Davidson SM, Payne Smith MD, MacCormac LP, Kahns S, Jensen PH, *et al.* (2004) Parkin is recruited into aggresomes in a stress-specific manner: over-expression of parkin reduces aggresome formation but can be dissociated from parkin's effect on neuronal survival. *Hum Mol Genet* **13**, 117-135.

Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, *et al.* (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* **25**, 302-305.

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Zhang Y, Gao J, Chung KK, Huang H, Dawson VL and Dawson TM (2000) Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci U S A* **97**, 13354-13359.

Application Reference:

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.

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