Parkin (human; full length), pAb

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

Cat. No.	**68-0018-100**	Quantity:	100 µg
Lot. No.	**30255**	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

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 (Muquit *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 (Shimura *et al.*, 2000; Muquit *et al.*, 2004). 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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human Parkin (residues 1-465) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Parkin at ~52 kDa

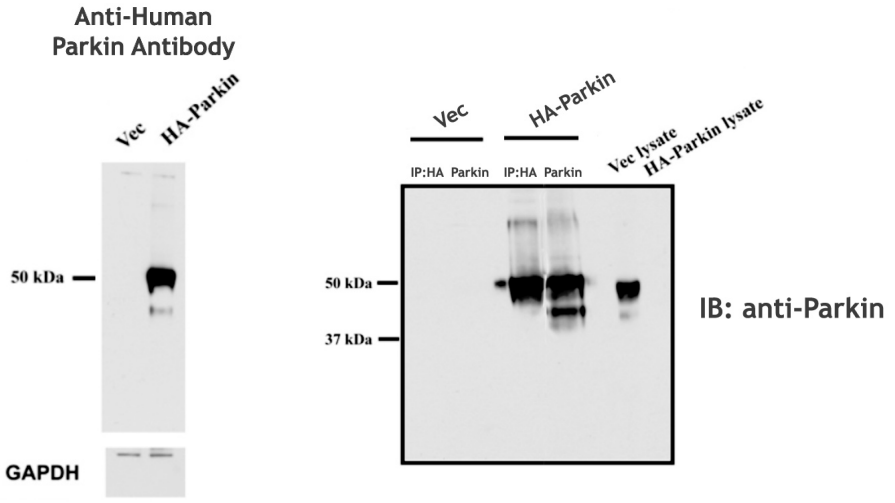
Reactivity: human, mouse and rat

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



Western Blotting Analysis:
By Western blotting, human Parkin was detected in lysates from HEK293 cells transfected with HA-Parkin compared to empty HA-vector (Vec) when probed with 1.0 µg/ml of anti-human Parkin antibody (Cat# 68-0018-100).

Immunoprecipitation:
HEK293 cells over-expressing human HA-Parkin or an empty HA-vector (Vec) were lysed. Immunoprecipitation was performed on cell lysates by capturing with 5 µl of HA-Agarose (Sigma) or protein G sepharose bound to 5.0 µg of anti-Parkin antibody (Cat# 68-0018-100). Immunoprecipitated protein was denatured in SDS and subjected to SDS-PAGE on an 8% gel and Western Blotting was carried out with 1.0 µg/ml of anti-Parkin antibody (Cat# 68-0018-100).

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

regulate its ubiquitin ligase activity (Yamamoto *et al.*, 2005). Parkin binds Ube2L6 through its c-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 endogenous, Parkin has been identified in aggresomes, co-localised with ubiquitin, however this has been shown to be variable, depending on the stress (Muquit *et al.*, 2004). PTEN Induced putative Kinase 1 (PINK1) has been shown to phosphorylate Parkin at a 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 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 (human) polyclonal antibody was raised in sheep against Parkin (residues 1-465 of human Parkin). 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 an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-Parkin (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

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Muqit MMK, Davidson SM, Smith MDP, MacCormac LP, Kahns S, Jensen PH, Wood NW, Latchman DS (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 Molec Genet* **13**, 117-135.

Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimuzu N, Iwai K, Chiba T, Tanaka K and Suzuki T (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin ligase. *Nature Genet* **25**, 302–305.

Yamamoto A, Friedlein A, Imai Y, Takahashi R, Kahle PJ, Haass C (2005) Parkin phosphorylation and modulation of its E3 ubiquitin ligase activity. *J Biol Chem* **280**, 3390-9.

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Application Reference:

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