Parkin [untagged]

E3 ligase

Alternate Names: PARK2, PRKN

Cat. No.	**63-0048-025**	Quantity:	25 µg
Lot. No.	**30340**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

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 kinase 1 (CK 1) suggesting that the phosphorylation of Parkin may act to regulate its ubiquitin ligase activity (Yamamoto *et al.*, 2004). Parkin binds Ube2L6 through its c-terminal domain and has been shown to auto-ubiquitylate leading to its own degradation (Zhang *et al.*, 2000). Par-

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 25 µg

Concentration: 0.67 mg/ml

Formulation: 50 mM Hepes pH 7.5, 150 mM NaCl, 2 mM DTT, 10% glycerol

Molecular Weight: ~51.6 kDa

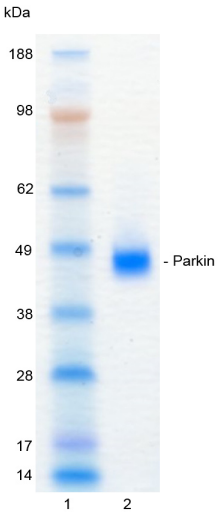
Purity: >90% by InstantBlue™ SDS-PAGE

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

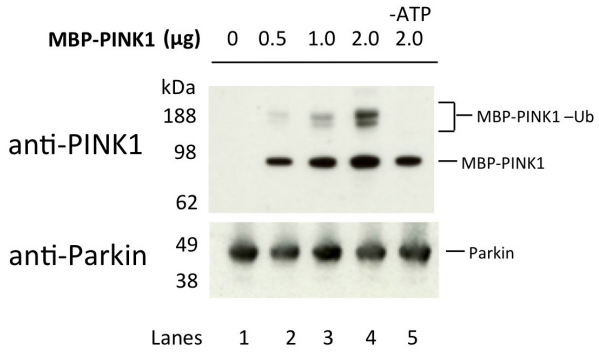
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Parkin



Protein Identification:
Confirmed by mass spectrometry.



E3 Ligase Activity Assay:
The activity of Parkin was validated through its ability to catalyse the generation of MBP-PINK1 ubiquitin conjugates (after the activation of Parkin via its phosphorylation by MBP-PINK1). MBP-PINK1 (0, 0.5, 1.0 and 2.0 µg) was incubated in kinase assay buffer with Parkin (2.0 µg) in the presence or absence of ATP for 60 minutes at 30˚C. The ubquitylation reactions were then initiated through the addition of His-UBE1, the E2 conjugating enzyme His-UBE2L3 (UbcH7) and ubiquitin; the reactions were then incubated for a further 60 minutes at 30˚C. MBP-PINK1 ubiquitin conjugates were identified by Western blotting using an anti-PINK1 antibody (lanes 3 and 4) which were observed in the presence of ATP and MBP-PINK1. Ubiquitin conjugates were not observed in the absence of MBP-PINK1 (lane 1) or ATP (lane 5).

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

kin 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 (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 phos phorylating 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); active Parkin may then ubiquitylate and tag damaged mitochondria for clearance by mitophagy. USP30 (a deubiquitylase (DUB) localized to mitochondria) antagonizes mitophagy driven by Parkin and PINK1 by removing ubiquitin attached by Parkin onto damaged mitochondria preventing 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:

Asakawa S, Tsunematsu K., Takayanagi A, Sasaki T, Shimizu A, Shintani A, Kawasaki K, Mungall AJ, Beck S, Minoshima S, Shimizu N (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* **510**, 370-5.

Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R (2001) An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of parkin. *Cell* **105**, 891-902.

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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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Physical Characteristics

Continued from page 1

Protein Sequence:

MIVFVRFNSSHGFPVEVDSDTSIFQLKEVVAKR
QGVPADQLRVIFAGKELRNDWTVQNCDLDQQSI
VHIVQRPWRKGQEMNATGGDDPRNAAGGCEREP
QSLTRVDLSSSVLPGDSVGLAVILHTDSRKDSP
PAGSPAGRSIYNSFYVYCKGPCQRVQPGKLRVQ
CSTCRQATLTLTQGPSCWDDVLIPNRMSGECQS
PHCPGTSAEFFFKCGAHPTSDKETSVALHLIAT
NSRNITCITCTDVRSPVLVFQCNSRHVICLDCF
HLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNSL
IKELHHFRILGEEQYNRYQQYGAEECVLQMGGV
LCPRPGCGAGLLPEPDQRKVTCEGGNGLGCGFA
FCRECKEAYHEGECSAVFEASGTTTQAYRVDER
AAEQARWEAASKETIKKTTKPCPRCHVPVEKNG
GCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHW
FDV

Parkin (regular text): Start ***bold italics***
(amino acid residues 1-465)
Accession number: NP_004553.2

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