

Enabling Parkin Research

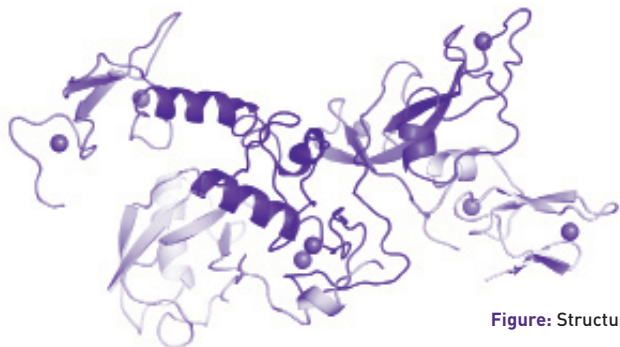


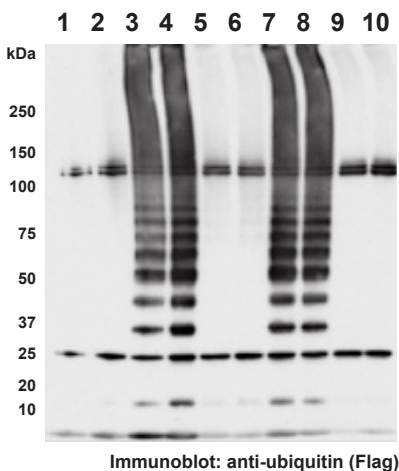
Figure: Structure of human Parkin (adapted from Spratt *et al.*, 2014)

Data supports a model in which the optimal activation of Parkin is regulated by a dual mechanism requiring both phosphorylation of its Ubl domain at Ser65 and the binding to Parkin of ubiquitin phosphorylated at Ser65. Critically Ser65 phosphorylation of both Parkin and ubiquitin is regulated by the kinase PINK1 (Kane *et al.*, 2014; Kazlauskaitė *et al.*, 2014; Koyano *et al.*, 2014).

In support of further research to follow up on these exciting discoveries Ubiquigent is pleased to announce that in collaboration with UbiQ Bio BV and the University of Dundee we have added the following PINK1/Parkin signalling-related phosphorylated ubiquitins to our extensive portfolio of reagents.

Synthetic ubiquitin phosphorylated on Ser65 (ubiquitin (pSer65)) activates Parkin E3 ligase-mediated ubiquitylation

Full-length Parkin (2 µg; Cat# 63-0048-025) was incubated at 30 °C with the ubiquitylation assay components Ube1 (0.1 µM; Cat# 61-0001) and Ube2L3 (1 µM; Cat# 62-0042) in the presence of 50 µM ubiquitin (comprising 20 µg of FLAG-ubiquitin mixed with nothing (lanes 1 and 2) or 5 µg of either enzymatically made ubiquitin (pSer65) (lanes 3 and 4), ubiquitin (lanes 5 and 6), synthetically made ubiquitin (pSer65) (Cat# 60-0202-050) (lanes 7 and 8) synthetically made ubiquitin (Cat# 60-0200-050) (lanes 9 and 10). Reactions were terminated after 60 min by the addition of Lithium Dodecyl Sulfate (LDS) loading buffer and products were analysed by Sodium Dodecyl Sulfate (SDS) PAGE followed by immunoblotting. Ubiquitin was detected using an anti-FLAG antibody.



Immunoblot: anti-ubiquitin (Flag)

Data generated and kindly provided by A. Kazlauskaitė from the Muqit lab at the MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, Scotland, U.K. See Kazlauskaitė *et al.* (2014) for details regarding how ubiquitin (pSer65) has been demonstrated to activate the E3 ligase Parkin.

NEW Parkin-Related Phospho-Ubiquitins

Description	Cat. #	Size	Price
Ubiquitin (pSer65)	60-0202-050	50 µg	£150 €180
Biotin-Ahx-Ubiquitin (pSer65)	60-0207-050	50 µg	£183 €220
Ubiquitin (synthetic)	60-0200-050	50 µg	£67 €80
Bio-Ahx-Ubiquitin (synthetic)	60-0201-050	50 µg	£83 €100

Antibodies

Description	Cat. #	Size	Price
Parkin (human; full length), pAb*	68-0018-100	100 µg	£215 €259
Parkin pSer65 (human; residues 60 - 72), pAb*	68-0056-100	100 µg	£215 €259
PINK1 (human; residues 175 - 250), pAb*	68-0019-100	100 µg	£215 €259
PINK1 pThr257 (human; residues 250 - 262), pAb*	68-0057-100	100 µg	£215 €259

Recombinant Proteins

Description	Cat. #	Size	Price
Parkin	63-0048-025	25 µg	£295 €356
PINK1	66-0043-050	50 µg	£295 €356
PINK1 (D359A)	66-0044-050	50 µg	£295 €356
USP30 CD(57-517)	64-0057-050	50 µg	£285 €345

References:

Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA *et al.* (2014) PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol* 205, 143-153.

Kazlauskaitė 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.

Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M *et al.* (2014) Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 510, 162-166.

Spratt DE, Walden H, Shaw GS (2014) RBR E3 ubiquitin ligases: new structures, new insights, new questions. *Biochem J* 458, 421-37.

UbiQ

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