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MATERIAL DATA SHEET

Parkin, *human recombinant*
Cat. # E3-160

The E3 Ubiquitin ligase Parkin (encoded by the PARK2 gene) is an essential part of the cellular machinery that participates in the removal of damaged mitochondria. Mutations in PARK2 are known to cause a form of Parkinson's disease known as autosomal recessive juvenile Parkinson's disease (AR-JP), and the mechanisms by which defective Parkin ligase contributes to the dopaminergic cell death in this disease is an area of intense investigation.

Reported substrates for Parkin include BCL2, GPR37, MIRO1, MFN1, MFN2, TOMM20, USP30, and many others. Parkin (an RBR-class Ubiquitin ligase) structures have recently been reported by multiple groups, and reveal that the ligase is folded upon itself to produce an auto-inhibited state. The auto-inhibition is relieved by interactions with PINK1 kinase (which can phosphorylate both Parkin and Ubiquitin at serine residue number 65) and pS65 phospho-Ubiquitin by mechanisms that are under investigation.

In vitro, Parkin may be activated by treatment with recombinant PINK1, or addition of low concentrations of pS65-phosphoubiquitin. Parkin has been reported to generate poly-Ubiquitin chains in K6, K11, K48, and K63 linkages both *in vitro* and *in vivo*. This recombinant protein is untagged.

Product Information	
Quantity:	25 µg
Stock:	X mg/ml (X µM) in 25 mM Tris-HCl pH 8.5, 200 mM NaCl, 0.03% Brij35, 10% (v/v) Glycerol, 5 mM TCEP
MW:	52 kDa
Purity:	≥ 90% by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie Blue Stain.

Use & Storage	
Use:	Reaction conditions will need to be optimized for each specific application. As supplied, Parkin has negligible E3 ligase activity as determined by the lack of autoubiquitination in an *in vitro* assay. Parkin ligase activity is greatly enhanced by phosphorylation with PINK1 kinase (**AP-180**) or by the addition of low concentrations of phosphorylated Ubiquitin (**U-102**).
Storage:	Store at -80°C. Avoid multiple freeze/thaw cycles.

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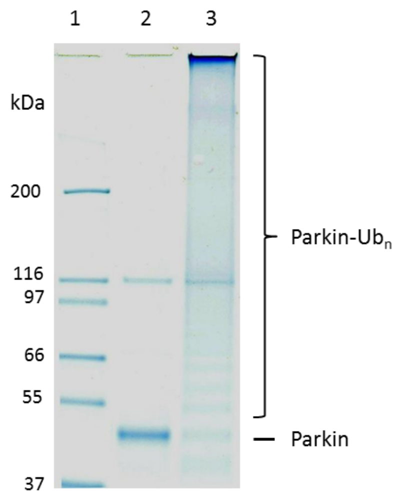
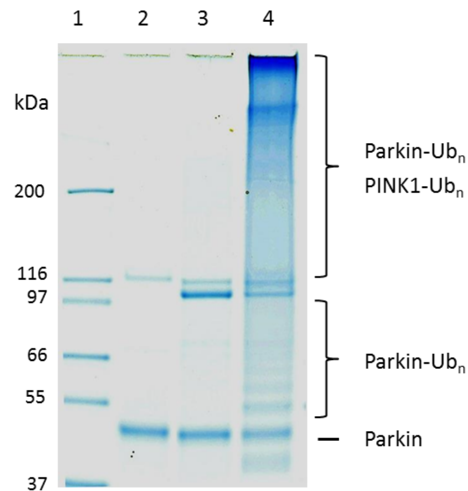
Example Data

Activation of Parkin Using pS65-Ubiquitin



Lane 1: Molecular Weight Markers
Lane 2: Parkin Reaction without PINK1
Lane 3: Parkin Reaction without PINK1, +pS65-Ub

Activation of Parkin Using PINK1 kinase



Lane 1: Molecular Weight Markers
Lane 2: Parkin Reaction without PINK1
Lane 3: Parkin Reaction with PINK1, -ATP
Lane 4: Parkin Reaction with PINK1, +ATP

Activation of Parkin Using pS65-Ubiquitin. Lane 1: Molecular Weight Markers. Lane 2: An *in vitro* assay was run with 50 nM E1, 1 µM E2, 1 µM Parkin, 50 µM Ubiquitin and 10 mM ATP for 60 minutes at 37°C. Lane 3: A similar reaction was done, supplemented with 5 µM pS65-Ubiquitin (**U-102**) in addition to the 50 µM unmodified Ubiquitin. No activity is seen in the absence of pS65-Ubiquitin, while significant autoubiquitination of Parkin is observed when pS65-Ubiquitin is added to the reaction at low concentration.

Activation of Parkin Using PINK1 kinase. Lane 1: Molecular Weight Markers. Lane 2: An *in vitro* assay was run with 50 nM E1, 1 µM E2, 1 µM Parkin, 50 µM Ubiquitin and 10 mM ATP for 120 minutes at 37°C. Lane 3: Reaction contained 50 nM E1, 1 µM E2, 1 µM Parkin, 0.5 µM PINK1 kinase (**AP-180**) and 50 µM Ubiquitin. No ATP was added. Lane 4: A 2-step *in vitro* assay was run. The first incubation step contained 1 µM Parkin, 0.5 µM PINK1 kinase and 10 mM ATP for 60 minutes at 37°C. 50 nM E1, 1 µM E2, 1 µM Parkin and 50 µM Ubiquitin were then added and the reaction continued for another 60 minutes at 37°C.

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Literature	
References:	Bingol, B. *et al.* (2014) Nature **510**: 370 Ordureau, A. *et al.* (2014) Mol. Cell **56**: 360 Riley, B.E. *et al.* (2013) Nat. Comm. **4**: doi:10.1038/ncomms2982 Saraff, S.A. et al. (2013) Nature **496**: 372 Spratt, D.E. *et al.* (2013) Nat. Comm. **4**: doi:10.1038/ncomms2983 Trempe, J.F. *et al.* (2013) Science **340**: 1451 Wauer T. *et. al.* (2015) Nature **524**: 370 Wauer T. & Komander, D. (2013) EMBO J **32**: 2099

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