Lot # XXXXX

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MATERIAL DATA SHEET

PINK1, *T. castaneum recombinant*
Cat. # AP-180

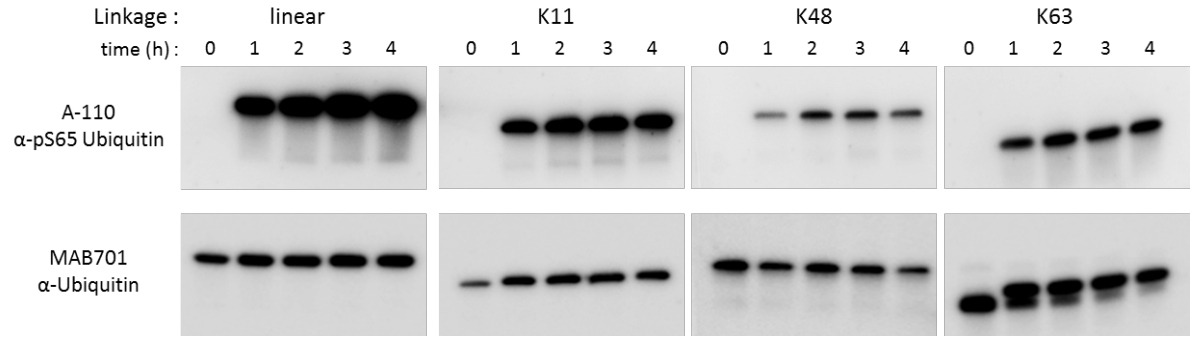
Serine/Threonine kinase PINK1 (PTEN-induced putative kinase protein 1) plays a critical role in preventing mitochondrial dysfunction during cellular stress. PINK is translated in the cytosol, then translocated to the outer mitochondrial membrane where it is rapidly cleaved and degraded as a part of normal mitochondrial function. In damaged (depolarized) mitochondria PINK becomes stabilized and accumulates, resulting in the subsequent phosphorylation of numerous proteins on the mitochondrial surface including Mfn2. Ultimately PARK2 (E3 Ubiquitin Ligase Parkin) is recruited to the damaged mitochondria where it is activated by PINK-mediated phosphorylation of PARK2 at serine 65, and PARK2 interaction with phosphorylated Ubiquitin (also phosphorylated by PINK on serine 65). This signaling cascade is critical for clearing the damaged mitochondria via selective autophagy (mitophagy) by mediating activation and translocation of PARK2.
Recombinant human PINK1 is not active *in vitro*, while this protein from the Red Flour Beetle (*Tribolium castaneum*) effectively phosphorylates recombinant Parkin, mono-Ubiquitin, and poly-Ubiquitin chains. It specifically phosphorylates both Parkin and Ubiquitin at serine 65. This recombinant protein contains N-terminal 6-His and MBP tags.

Product Information	
Quantity:	100 µg
Stock:	X mg/ml (X µM) in 20 mM HEPES pH 7.6, 50 mM NaCl, 10% (v/v) Glycerol, 1 mM TCEP
MW:	108 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. We recommend an initial PINK1 concentration of 0.5-2 µM for the phosphorylation of recombinant Parkin, Ubiquitin, or Polyubiquitin chains. See Example Data.
Storage:	Store at -80°C. Avoid multiple freeze/thaw cycles.

840 Memorial Drive, Cambridge, MA 02139 Phone: 617-576-2210 FAX: 617-492-3565
www.bostonbiochem.com

Example Data



25 µg of tetraubiquitin of each indicated linkage type was incubated in a 50 µl reaction (15 µM) containing 1 µM PINK1 and 2 mM ATP for 0-4 hours. At indicated times a portion of each reaction was removed and terminated with SDS-PAGE sample buffer. SDS-PAGE gels (10-20%) were used to resolve approximately 150 ng of Ubiquitin tetramer from each reaction. Western Blots were developed using either α-phospho-Ubiquitin, pS65 (Cat# A-110, upper panels) or anti-Ubiquitin (Cat# MAB701, lower panel). Primary antibodies were used at 1 µg/ml in PBST + 0.5% BSA, while HRP-labeled secondary antibodies (α-rabbit for A-110, α-mouse for MAB701) were used at a 1:10,000 dilution in PBST + 0.5% BSA. Further details are available upon request.

Literature

References:

Kane L.A., *et al.* (2014) J. Cell Biol. **205**: 143
Matsuda N., *et al.* (2010) J. Cell Biol. **189**: 211
Ordureau A., *et al.* (2014) Mol. Cell **56**: 360
Vives-Bauza C., *et al.* (2010) Proc. Natl. Acad. Sci. **107**: 378

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