Lot # XXXXX

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MATERIAL DATA SHEET

Phospho-Ubiquitin (pS65), Affinity purified pAb, Cat. # A-110

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.

Product Information	
Quantity:	50 µg
Source:	Rabbit Polyclonal
Antigen:	Peptide sequence from Ubiquitin, phosphorylated at Serine 65. Accession Number P0CG47
Purification:	Affinity purified against antigen
Stock:	0.5 mg/mL in PBS, pH 7.4, 50% glycerol

Use & Storage	
Specificity:	This antibody detects mono- and Polyubiquitin chains that are phosphorylated on Serine 65. It has no cross-reactivity with non-phosphorylated Ubiquitin, Ubiquitin phosphorylated at Serine 57 or Tyrosine 59, or phosphorylated Parkin.
Use:	Recommended concentration for Western Blot applications is 0.5- 1 µg/ml.
Storage:	Store at -20°C. Storage at -80°C is not recommended.

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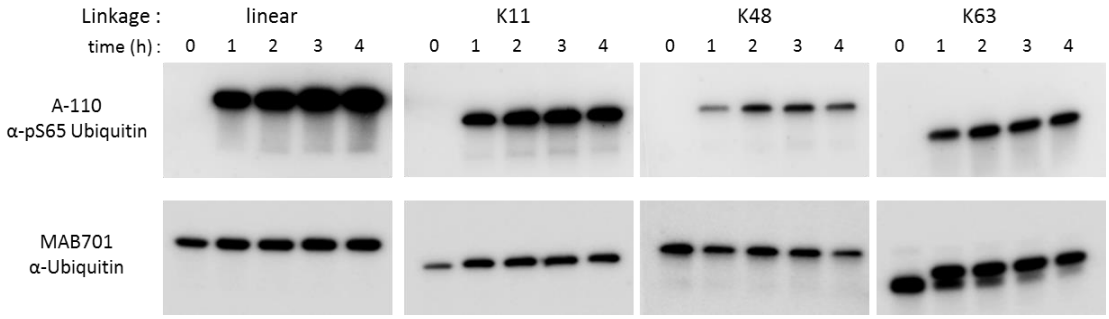
Example Data



Tetraubiquitin chains of each indicated linkage type were incubated for 0-4 hours in reactions containing recombinant PINK1 kinase (Cat# AP-180) and ATP. 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 (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:

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Matsuda N., *et al.* (2010) J. Cell Biol. **189**: 211
Ordureau A., *et al.* (2014) Mol Cell. **56**: 360
Ordureau A., *et al.* (2015) Mol. Cell **58** : 660
Vives-Bauza C., *et al.* (2010) Proc. Natl. Acad. Sci. **107**: 378
Wauer T., *et al.* (2015) EMBO J. **34**: 307

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