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Human Phospho-Ubiquitin (S65) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: A-110

DESCRIPTION

Species Reactivity	Human
Specificity	This antibody detects mono- and poly-Ubiquitin 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. Detects only recombinant, phosporylated Ubiquitin. Not recommended on natural lysates (see western blot results below).
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Peptide sequence from Ubiquitin, phosphorylated at Serine 65. Accession # P0CG47
Formulation	0.5 mg/ml in PBS pH 7.4, 50% (v/v) Glycerol See Certificate of Analysis for details.

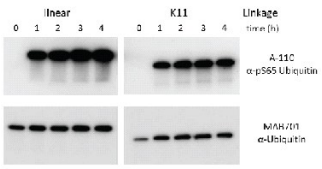
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5-1 µg/mL	See Below

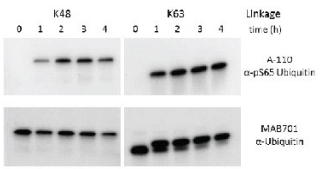
DATA

Western Blot



Detection of Phospho-Ubiquitin (S65) by Western blot. 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.

Western Blot



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PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Store at -20°C. Storage at -80°C is not recommended.

BACKGROUND

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.

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