Revision 1

Store at -20C

#37642

Phospho-Ubiquitin (Ser65) Antibody

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP	H	Endogenous	Rabbit	#P62987, #P0CG48, #P0CG47, #P62979	7311, 7316, 7314, 6233

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-Ubiquitin (Ser65) Antibody recognizes endogenous levels of ubiquitin protein only when phosphorylated at Ser65.

Species predicted to react based on 100% sequence homology

Mouse, Rat

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Ser65 of human ubiquitin protein. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Ubiquitin is a conserved polypeptide unit that plays an important role in the ubiquitin-proteasome pathway. Ubiquitin can be covalently linked to many cellular proteins by the ubiquitination process, which targets proteins for degradation by the 26S proteasome. Three components are involved in the target protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thiolester complex with the activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2, then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH_2 of the target protein lysine residue (1-3). The ubiquitin-proteasome pathway has been implicated in a wide range of normal biological processes and in disease-related abnormalities. Several proteins such as IκB, p53, cdc25A, and Bcl-2 have been shown to be targets for the ubiquitin-proteasome process as part of regulation of cell cycle progression, differentiation, cell stress response, and apoptosis (4-7).

Ubiquitin is phosphorlated at Ser65 by PINK1, leading to activation of the E3 ubiquitin ligase Parkin (8, 9). PINK1 accumulates on depolarized mitochondria, resulting in phosphorylation of ubiquitin and activation of Parkin, which then triggers the mitophagy pathway to clear damaged mitochondria. Loss-of-function mutations in PINK1 and Parkin result in early onset Parkinson's disease (10, 11).

Background References

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3. Hochstrasser, M. (2000) *Science* 289, 563-4.
4. Bernardi, R. et al. (2000) *Oncogene* 19, 2447-54.
5. Aberle, H. et al. (1997) *EMBO J* 16, 3797-804.
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7. Jesenberger, V. and Jentsch, S. (2002) *Nat Rev Mol Cell Biol* 3, 112-21.
8. Kane, L.A. et al. (2014) *J Cell Biol* 205, 143-53.
9. Koyano, F. et al. (2014) *Nature* 510, 162-6.
10. Kitada, T. et al. (1998) *Nature* 392, 605-8.
11. Valente, E.M. et al. (2004) *Science* 304, 1158-60.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: Human

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