Store at -20°C

#5621

K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb

☑ 100 µl
(10 western blots)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #6233
Swiss-Prot Acc. #P62988

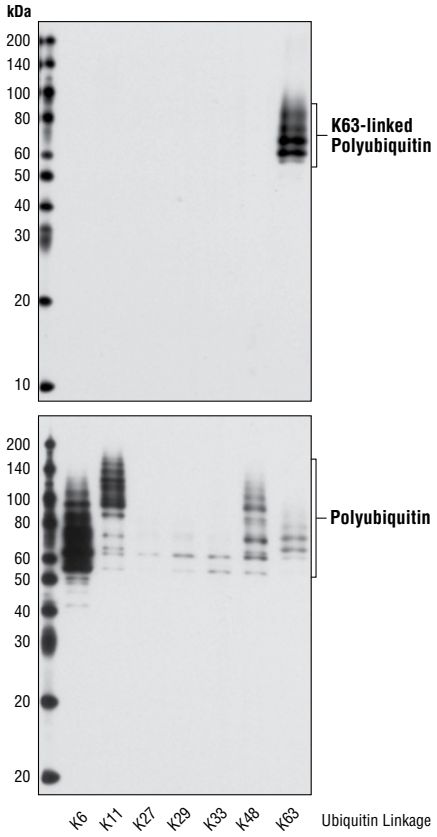
Applications	Species Cross-Reactivity*	Isotype
W Endogenous	All	Rabbit IgG**

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).

Substrate proteins are linked to ubiquitin using seven distinct ubiquitin lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63). Formation of a polyubiquitin chain occurs when a lysine residue of ubiquitin is linked to the carboxy-terminal glycine of another ubiquitin. Proteins polyubiquitinated at specific lysine residues display a tendency to be targeted for different processes (8). K63-linked polyubiquitin chains exert nonproteolytic functions in vivo, such as protein trafficking, kinase/phosphatase activation, and DNA damage control, all of which might be important in regulation of cancer survival and development (9,10).

Specificity/Sensitivity: K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb detects polyubiquitin chains formed by Lys63 residue linkage. It does not react with monoubiquitin or polyubiquitin chains formed by linkage to a different lysine residue.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the Lys63 branch of the human diubiquitin chain.



Western blot analysis of seven distinct recombinant polyubiquitin chains (300 ng each) using K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb (upper) and Ubiquitin Antibody #3933 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Ciechanover, A. (1998) *EMBO J.* 17, 7151-7160.
(2) Hochstrasser, M. (2000) *Nat. Cell Biol.* 2, E153-E157.
(3) Hochstrasser, M. (2000) *Science* 289, 563-564.
(4) Bernardi, R. et al. (2000) *Oncogene* 19, 2447-2454.
(5) Aberle, H. et al. (1997) *EMBO J.* 16, 3797-3804.
(6) Salomoni, P. and Pandolfi, P.P. (2002) *Nat. Cell Biol.* 4, E152-E153.
(7) Jesenberger, V. and Jentsch, S. (2002) *Nat. Rev. Mol. Cell Biol.* 3, 112-121.
(8) Komander, D. (2009) *Biochem Soc Trans* 37, 937-53.
(9) Chen, Z.J. and Sun, L.J. (2009) *Mol Cell* 33, 275-86.
(10) Yang, W.L. et al. (2010) *Oncogene* 29, 4493-503.

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

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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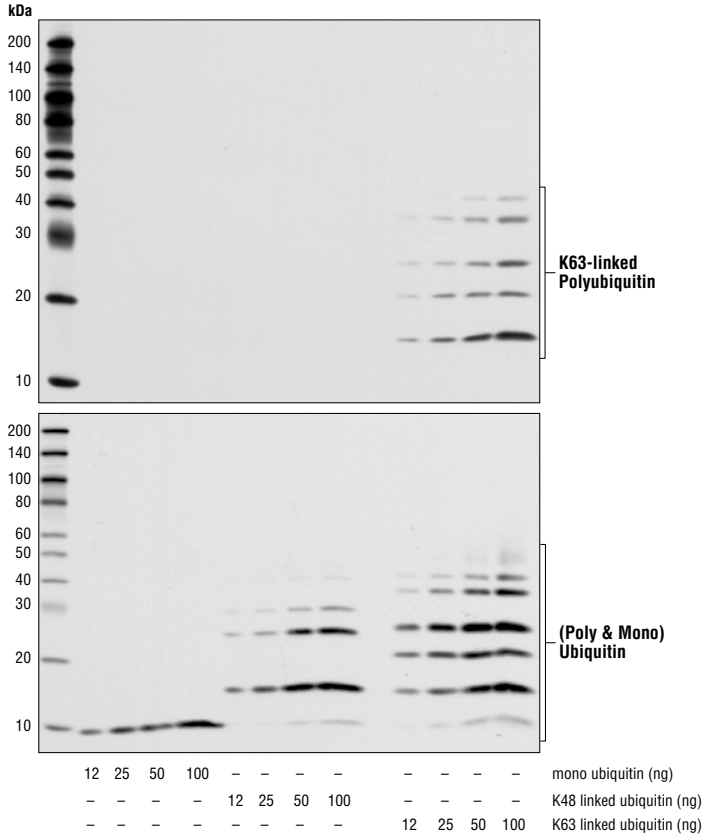
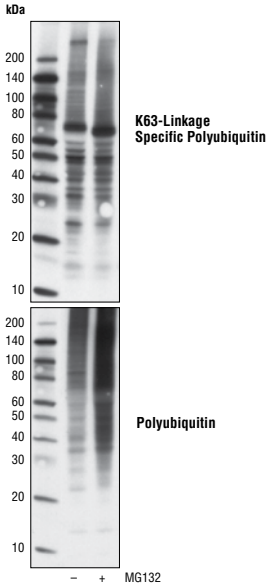
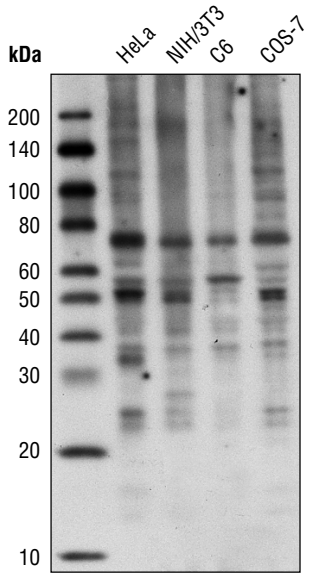


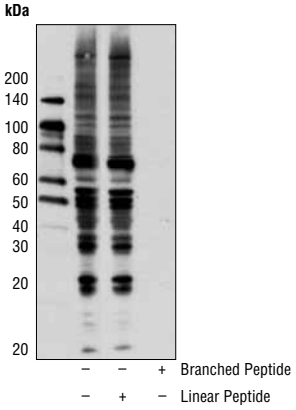
Western blot analysis comparing the titration of recombinant monoubiquitin, K48-linked polyubiquitin and K63-linked polyubiquitin using K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb (upper) and Ubiquitin Antibody #3933 (lower).



Western blot analysis of extracts from HeLa cells, untreated or treated with the proteasome inhibitor MG132 (10 µM for 6 hours), using K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb and Ubiquitin Antibody #3933 (lower).



Western blot analysis of various cell lines using K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb.



Western blot analysis of extracts from HeLa cells using K63-linkage Specific Polyubiquitin (D7A11) Rabbit mAb, untreated or following antibody pre-incubation with either K63 ubiquitinylated branched peptide to block the signal or a linear peptide surrounding K63 of ubiquitin that cannot block the signal.

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