#8081 Store at -20°C

K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb

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For Research Use Only. Not For Use In Diagnostic Procedures.

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

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

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.*

***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.

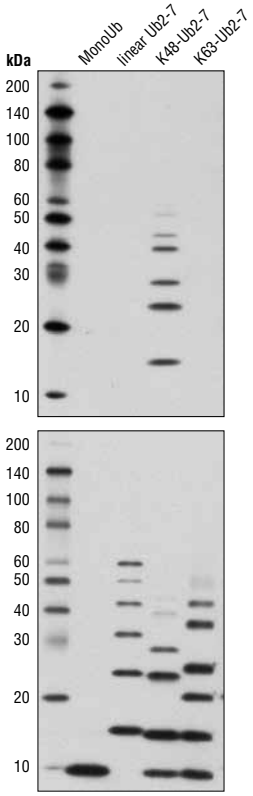
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background: 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).

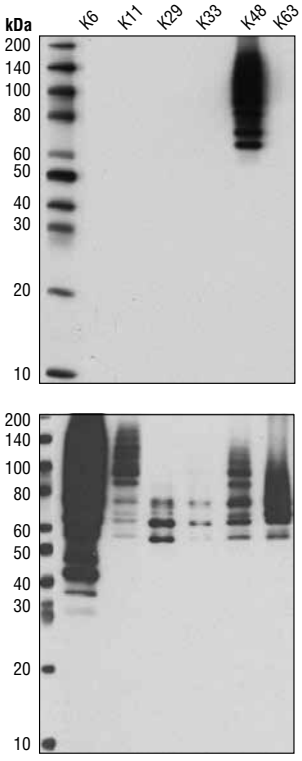
Substrate proteins are linked to ubiquitin using seven distinct ubiquitin lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63). Formation of a polyubiquitin chain occurs when a lysine residue of ubiquitin is linked to the carboxy-terminal glycine of another ubiquitin. Proteins polyubiquinated at specific lysine residues display a tendency to be targeted for different processes; K48-linked polyubiquitin chains mainly target proteins for proteasomal degradation while K63-linked polyubiquitin regulates protein function, subcellular localization, or protein-protein interactions (8).

Specificity/Sensitivity: K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb detects polyubiquitin chains formed by Lys48 residue linkage. This antibody does not react with monoubiquitin or polyubiquitin chains formed by specific linkage to a different lysine residue.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the Lys48 branch of the human diubiquitin chain.



Western blot analysis of recombinant monoubiquitin (MonoUb), linear unbranched ubiquitin chain (Ub2-7), K48-linked polyubiquitin (K48-Ub2-7) and K63-linked polyubiquitin (K63-Ub2-7), using K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb (upper) and Ubiquitin (P4D1) Mouse mAb #3936 (lower). Ubiquitin chains range from 2 to 7 in length.



Western blot analysis of six distinct recombinant polyubiquitin chains using K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb (upper) and Ubiquitin (P4D1) Mouse mAb #3936 (lower).

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.

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.

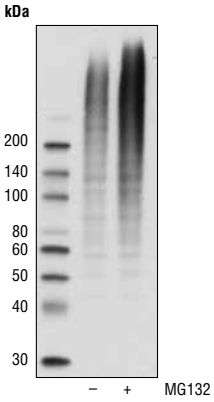
(4) Bernardi, R. et al. (2000) *Oncogene* 19, 2447-2454.

(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.



Western blot analysis of H1703 cells, untreated or treated with MG132 as indicated, using K48-linkage Specific Polyubiquitin (D9D5) Rabbit mAb.