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#14993

# MLKL (D2I6N) Rabbit mAb

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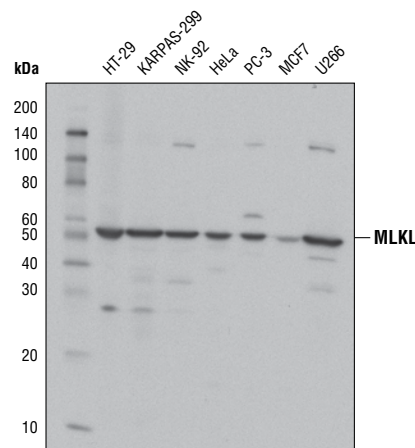
Applications W Endogenous	Species Cross-Reactivity* H	Molecular Wt. 54 kDa	Isotype Rabbit IgG**
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**Background:** Necroptosis, a regulated pathway for necrotic cell death, is triggered by a number of inflammatory signals including cytokines in the tumor necrosis factor (TNF) family, pathogen sensors such as toll-like receptors (TLRs), and ischemic injury (1,2). The process is negatively regulated by caspases and is initiated through a complex containing the RIP1 and RIP3 kinases, typically referred to as the necrosome. Mixed lineage kinase domain-like protein (MLKL) is a pseudokinase that was identified as downstream target of RIP3 in the necroptosis pathway (3,4). During necroptosis RIP3 is phosphorylated at Ser227, which recruits MLKL and leads to its phosphorylation at Thr357 and Ser358 (3). Knockdown of MLKL through multiple mechanisms results in inhibition of necroptosis (3-5). While the precise mechanism for MLKL-induced necroptosis is unclear, some studies have shown that necroptosis leads to oligomerization of MLKL and translocation to the plasma membrane, where it effects membrane integrity (6-9).

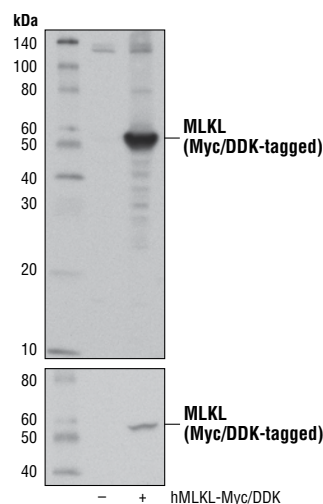
**Specificity/Sensitivity:** MLKL (D2I6N) Rabbit mAb recognizes endogenous levels of total MLKL protein. This antibody also cross-reacts with an unidentified protein of 130 kDa in some cell lines.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human MLKL protein.

Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with a construct expressing Myc/DDK-tagged full-length human MLKL protein (hMLKL-Myc/DDK; +), using MLKL (D2I6N) Rabbit mAb (upper) and Myc-Tag (71D10) Rabbit mAb #2278 (lower).



Western blot analysis of extracts from various cell lines using MLKL (D2I6N) Rabbit mAb. KARPAS cell Line source: Dr. Abraham Karpas at the University of Cambridge.



**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 product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com)

**Background References:**

- (1) Christofferson, D.E. and Yuan, J. (2010) *Curr Opin Cell Biol* 22, 263-8.
- (2) Kaczmarek, A. et al. (2013) *Immunity* 38, 209-23.
- (3) Sun, L. et al. (2012) *Cell* 148, 213-27.
- (4) Wang, Z. et al. (2012) *Cell* 148, 228-43.
- (5) Wu, J. et al. (2013) *Cell Res* 23, 994-1006.
- (6) Cai, Z. et al. (2014) *Nat Cell Biol* 16, 55-65.
- (7) Chen, X. et al. (2014) *Cell Res* 24, 105-21.
- (8) Wang, H. et al. (2014) *Mol Cell* 54, 133-46.
- (9) Dondelinger, Y. et al. (2014) *Cell Rep* 7, 971-81.

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**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.**

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