Revision 1

Store at -20C

#14516

Phospho-MLKL (Thr357/Ser358) Antibody

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W	H	Endogenous	54	Rabbit	#Q8NB16	197259

Product Usage Information

Application	Dilution
Western Blotting	1:1000

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-MLKL (Thr357/Ser358) recognizes endogenous levels of MLKL protein only when phosphorylated at Thr357 and Ser358. This antibody can recognize single phosphorylation sites at Thr357 or Ser358, or when dually phosphorylated at both sites.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr357/Ser358 of human MLKL protein. Antibodies are purified by protein A and peptide affinity chromatography.

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 a 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 affects membrane integrity (6-9).

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.

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

Cross-Reactivity Key

H: Human

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Limited Uses

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