Revision 3

Store at -20C

#37333

Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb

Cell Signaling TECHNOLOGY®

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

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IF-IC	M	Endogenous	54	Rabbit IgG	#Q9D2Y4	74568

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:1600

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.

For a carrier free (BSA and azide free) version of this product see product #17825.

Specificity/Sensitivity

Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb recognizes endogenous levels of mouse MLKL protein only when phosphorylated at Ser345. Weak, non-specific nuclear staining has been observed by immunofluorescence (IF-IC).

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Ser345 of mouse MLKL protein.

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 **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

M: Mouse

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