Revision 3

Store at -20C

#44590

Phospho-RIP (Ser166) (D8I3A) Rabbit mAb

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IF-IC, FC-FP	H	Endogenous	78-82	Rabbit IgG	#Q13546	8737

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:400
Flow Cytometry (Fixed/Permeabilized)	1:200 - 1:800

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 #96323.

Specificity/Sensitivity

Phospho-RIP (Ser166) (D8I3A) Rabbit mAb (IF Preferred) recognizes endogenous levels of RIP protein only when phosphorylated at Ser166. This antibody is preferred for immunofluorescence whereas Phospho-RIP (Ser166) (D1L3S) Rabbit mAb #65746 is preferred for western blot. Weak centriolar background staining was observed in some cell types.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Ser166 of human RIP protein.

Background

The receptor-interacting protein (RIP) family of serine-threonine kinases (RIP, RIP2, RIP3, and RIP4) are important regulators of cellular stress that trigger pro-survival and inflammatory responses through the activation of NF-κB, as well as pro-apoptotic pathways (1). In addition to the kinase domain, RIP contains a death domain responsible for interaction with the death domain receptor Fas and recruitment to TNF-R1 through interaction with TRADD (2,3). RIP-deficient cells show a failure in TNF-mediated NF-κB activation, making the cells more sensitive to apoptosis (4,5). RIP also interacts with TNF-receptor-associated factors (TRAFs) and can recruit IKKs to the TNF-R1 signaling complex via interaction with NEMO, leading to IκB phosphorylation and degradation (6,7). Overexpression of RIP induces both NF-κB activation and apoptosis (2,3). Caspase-8-dependent cleavage of the RIP death domain can trigger the apoptotic activity of RIP (8).
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 (9,10). The process is negatively regulated by caspases and is initiated through a complex containing the RIP and RIP3 kinases, typically referred to as the necrosome. Necroptosis is inhibited by a small molecule inhibitor of RIP, necrostatin-1 (Nec-1) (11). Research studies show that necroptosis contributes to a number of pathological conditions, and Nec-1 has been shown to provide neuroprotection in models such as ischemic brain injury (12). RIP is phosphorylated at several sites within the kinase domain that are sensitive to Nec-1, including Ser14, Ser15, Ser161, and Ser166 (13).

Background References

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5. Kelliher, M.A. et al. (1998) *Immunity* 8, 297-303.
6. Devin, A. et al. (2000) *Immunity* 12, 419-29.
7. Zhang, S.Q. et al. (2000) *Immunity* 12, 301-11.
8. Lin, Y. et al. (1999) *Genes Dev* 13, 2514-26.
9. Christofferson, D.E. and Yuan, J. (2010) *Curr Opin Cell Biol* 22, 263-8.
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11. Degterev, A. et al. (2008) *Nat Chem Biol* 4, 313-21.
12. Degterev, A. et al. (2005) *Nat Chem Biol* 1, 112-9.
13. Ofengeim, D. and Yuan, J. (2013) *Nat Rev Mol Cell Biol* 14, 727-36.

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) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human

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