Revision 4

Store at -20C

#31122

Phospho-RIP (Ser166) 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

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

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W	M	Endogenous	80	Rabbit	#Q60855	19766

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-RIP (Ser166) Antibody recognizes endogenous levels of mouse RIP protein only when phosphorylated at Ser166. A nonspecific band is observed at 22 kDa.

Species predicted to react based on 100% sequence homology

Rat

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser166 of mouse RIP protein. Antibodies are purified by protein A and peptide affinity chromatography.

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.
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10. Kaczmarek, A. et al. (2013) *Immunity* 38, 209-23.
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

Cross-Reactivity Key

M: Mouse

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