# RD SYSTEMS a biotechne brand

# Human/Mouse/Rat RIPK1/RIP1 Antibody

Monoclonal Mouse IgG<sub>1</sub> Clone # 334640 Catalog Number: MAB3585

DESCRIPTION		
Species Reactivity	Human/Mouse/Rat	
Specificity	Detects human, mouse and rat RIPK1/RIP1 in Western blots.	
Source	Monoclonal Mouse IgG <sub>1</sub> Clone # 334640	
Purification	Protein A or G purified from hybridoma culture supernatant	
Immunogen	<i>E. coli</i> -derived recombinant human RIPK1/RIP1 Met1-Asn671 Accession # Q13546	
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.	

### APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	8-25 μg/mL	See Below
Simple Western	10 µg/mL	See Below
Knockout Validated	RIPK1/RIP1 is spec in RIPK1/RIP1 knoc	cifically detected in MCF-7 human breast cancer cell line parental cell line but is not detectable skout MCF-7 cell line

### DATA



## Detection of

Human/Mouse/Rat RIPK1/RIP1 by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line, Jurkat human acute T cell leukernia cell line, DA3 mouse myeloma cell line, and L6 rat myoblast cell line. PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat RIPK1/RIP1 Monoclonal Antibody (Catalog # MAB3585) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for RIPK1/RIP1 at approximately 75 KDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer

#### Group 2.



Detection of Human RIPK1/RIP1 by Simple Western<sup>TM</sup>. Simple Western Iane view shows lysates of Jurkat human acute T cell leukemia cell line and MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for RIPK1/RIP1 at approximately 78 kDa (as indicated) using 10 ug/mL of Mouse Anti-Human/Mouse/Rat RIPK1/RIP1 Monoclonal Antibody (Catalog # MAB3585). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

#### Immunocytochemistry



RIPK1/RIP1 in MCF-7 Human Cell Line. RIPK1/RIP1 was detected in immersion fixed MCF-7 human breast cancer cell line using Mouse Anti-Human/Mouse/Rat RIPK1/RIP1 Monoclonal Antibody (Catalog # MAB3585) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

#### Knockout Validated



Western Blot Shows Human RIPK1/RIP1 Specificity by Using Knockout Cell Line. Western blot shows lysates of MCF-7 human breast cancer parental cell line and RIPK1/RIP1 knockout MCF-7 cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat RIPK1/RIP1 Monoclonal Antibody (Catalog # MAB3585) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for RIPK1/RIP1 at approximately 75 kDa (as indicated) in the parental MCF-7 cell line, but is not detectable in knockout MCF-7 cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Rev. 6/17/2019 Page 1 of 2



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## RDSYSTEMS a biotechne brand

Human/Mouse/Rat RIPK1/RIP1 Antibody

Monoclonal Mouse IgG<sub>1</sub> Clone # 334640 Catalog Number: MAB3585

PREPARATION AND STORAGE			
Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.		
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C		
Stability & Storage	<ul> <li>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</li> <li>12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>		

### BACKGROUND

Receptor-Interacting Protein 1 (RIP1, also known as RIPK1) is a 671 amino acid (aa) 75 kDa protein that contains an N-terminal protein kinase domain, a C-terminal death domain, and a unique internal region called the intermediate domain. RIP1 is a serine/threonine protein kinase and is constitutively expressed in many tissues. RIP1 interacts with the cytoplasmic death domain of FAS and TNF receptors and is an important element in the signal transduction machinery that mediates apoptosis. RIP1 has been shown to interact with a number of proteins including TRADD, TRAF1, TRAF2, and TRAF3, to form larger signaling complexes. These complexes, in turn, activate specific signaling cascades, such as NFkB. RIP1 also interacts through the C-terminal RIP homotypic interaction motif (RHIM) of TRIF in TLR3 dependent activation of NFkB.

Rev. 6/17/2019 Page 2 of 2



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