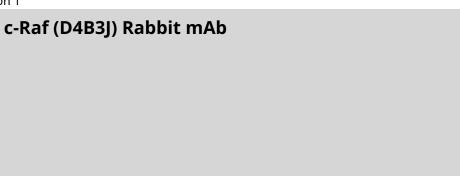
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Applications: W, IP	Reactivity: H M R	Sensitivity: Endogenous	MW (kDa): 75	Source/Isotype: Rabbit IgG	UniProt ID: #P04049	Entrez-Gene Id: 5894		
Product Usage Information		Application Western Blotting Immunoprecipitation			Dilution 1:1000 1:50			
Storage		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.						
Specificity/Sen	sitivity	c-Raf (D4B3J) Rabbit mAb recognizes endogenous levels of total c-Raf protein.						
Source / Purification Monoclonal antibody is produced by immunizing anim central region of human c-Raf protein.				unizing animals with re	combinant protein	specific with a		
Background	d A-Raf, B-Raf, and c-Raf (Raf-1) are the main effectors recruited by GTP-bound Ras to activate the MEK MAP kinase pathway (1). Activation of c-Raf is the best understood and involves phosphorylation at multiple activating sites, including Ser338, Tyr341, Thr491, Ser494, Ser497, and Ser499 (2). p21- activated kinase (PAK) has been shown to phosphorylate c-Raf at Ser338, and the Src family phosphorylates Tyr341 to induce c-Raf activity (3,4). Ser338 of c-Raf corresponds to similar sites in A- (Ser299) and B-Raf (Ser445), although this site is constitutively phosphorylated in B-Raf (5). Inhibitory 14-3-3 binding sites on c-Raf (Ser259 and Ser621) can be phosphorylated by Akt and AMPK, respectiv (6,7). While A-Raf, B-Raf, and c-Raf are similar in sequence and function, differential regulation has be observed (8). Of particular interest, B-Raf contains three consensus Akt phosphorylation sites (Ser364 Ser428, and Thr439) and lacks a site equivalent to Tyr341 of c-Raf (8,9). Research studies have shown that the B-Raf mutation V600E results in elevated kinase activity and is commonly found in malignan melanoma (10). Six residues of c-Raf (Ser29, Ser43, Ser289, Ser296, Ser301, and Ser642) become hyperphosphorylated in a manner consistent with c-Raf inactivation. The hyperphosphorylation of these six sites is dependent on downstream MEK signaling and renders c-Raf unresponsive to subsequent activation events (11).					sphorylation at 99 (2). p21- family imilar sites in A-Raf caf (5). Inhibitory AMPK, respectively egulation has been tion sites (Ser364, dies have shown und in malignant 42) become phorylation of		
Background Re	ferences	 Avruch, J. et al. (1994) <i>Trends Biochem Sci</i> 19, 279-83. Chong, H. et al. (2001) <i>EMBO J</i> 20, 3716-27. King, A.J. et al. (1998) <i>Nature</i> 396, 180-3. Fabian, J.R. et al. (1993) <i>Mol Cell Biol</i> 13, 7170-9. Mason, C.S. et al. (1999) <i>EMBO J</i> 18, 2137-48. Zimmermann, S. and Moelling, K. (1999) <i>Science</i> 286, 1741-4. Sprenkle, A.B. et al. (1997) <i>FEBS Lett</i> 403, 254-8. Marais, R. et al. (1997) <i>J Biol Chem</i> 272, 4378-83. Guan, K.L. et al. (2000) <i>J Biol Chem</i> 275, 27354-9. Davies, H. et al. (2002) <i>Nature</i> 417, 949-54. Dougherty, M.K. et al. (2005) <i>Mol Cell</i> 17, 215-24. 						
Species Reactiv	vity	Species reactivity is dete	ermined by testing	g in at least one approve	ed application (e.g.,	western blot).		
Western Blot B	uffer	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 Ke	≥y	W: Western Blotting IP: Immunoprecipitation						
Cross-Reactivit	у Кеу	H: Human M: Mouse R: Rat						
Trademarks an	d Patents	Cell Signaling Technolog	gy is a trademark	of Cell Signaling Techno	logy, Inc.			
		XP is a registered trade	mark of Cell Signa	ling Technology, Inc.				

KARPAS cell line source: Dr. Abraham Karpas at the University of Cambridge.

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