## Phospho-c-Raf (Ser338) (56A6) Rabbit mAb



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

W, W-S	Reactivity: H M R Mk	<b>Sensitivity:</b> Endogenous	<b>MW (kDa):</b> 74	<b>Source/Isotype:</b> Rabbit IgG	UniProt ID: #P04049	Entrez-Gene Id 5894
Product Usage Information		<b>Application</b> Western Blotting Simple Western™		<b>Dilution</b> 1:1000 1:50 - 1:250		
Storage		Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.				
Specificity/Sensitivity		Phospho-c-Raf (Ser338) (56A6) Rabbit mAb detects endogenous levels of c-Raf only when phosphorylated at Ser338.				
Source / Purification		Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding serine 338 of human Raf.				
Background		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-Raf (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, respectively (6,7). While A-Raf, B-Raf, and c-Raf are similar in sequence and function, differential regulation has been 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 malignant 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).				
		melanoma (10). Six re hyperphosphorylated these six sites is depe	on V600E results in esidues of c-Raf (Ser l in a manner consisendent on downstre	elevated kinase activity a 29, Ser43, Ser289, Ser29 stent with c-Raf inactivat	and is commonly fo 6, Ser301, and Ser6 ion. The hyperphos	dies have shown bund in malignant 542) become sphorylation of

**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 W-S: Simple Western™

Cross-Reactivity Key H: Human M: Mouse R: Rat Mk: Monkey

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