Phospho-c-Raf (Ser259) Antibody

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

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<td>H M R Mk X</td>
<td>Endogenous</td>
<td>74</td>
<td>Rabbit</td>
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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 and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

- Phospho-c-Raf (Ser259) Antibody detects endogenous levels of c-Raf only when phosphorylated at Ser259.

Species predicted to react based on 100% sequence homology:

- Chicken

Source / Purification

- Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser259 of human c-Raf. Antibodies are purified by protein A and peptide affinity chromatography.

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).

Background References


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

- **WB:** Western Blotting
- **IP:** Immunoprecipitation

Cross-Reactivity Key

- H: human
- M: mouse
- R: rat
- Hm: hamster
- Mk: monkey
- Vir: virus
- C: chicken
- Dm: D. melanogaster
- X: Xenopus
- Z: zebrafish
- B: bovine
- Pg: pig
- Sc: S. cerevisiae
- Ce: C. elegans
- Hr: horse
- GP: Guinea Pig
- Rab: rabbit
- All: all species expected

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