

## c-Raf [pS259] Antibody

CATALOG NO.: XBP-4074

### BACKGROUND:

The Raf family of serine/threonine-specific kinases is comprised of three members (A-Raf, B-Raf, and c-Raf) that play a critical role in regulating cell growth and differentiation, and couple growth factor receptor stimulation to nuclear transcription factors via the Ras/mitogen-activated protein kinase (MAPK) pathway. c-Raf kinase (also known as Raf-1) is a key 74 kDa signal transducer of multiple extracellular stimuli that is regulated by several pathways, and that once activated, phosphorylates MEK which in turn phosphorylates ERK. Serine 259 is one of the three constitutive phosphorylation sites of c-Raf in resting cells together with serine 43 and serine 621. Serine 259 is phosphorylated by PKA and is the main mediator of PKA-induced inhibition of c-Raf kinase activity. Phosphorylation of serine 259 blocks the membrane localization of c-Raf.

### SPECIFICITY:

Human c-Raf. Mouse and rat (100% homologous) c-Raf have not been tested, but are expected to react. A-Raf (90%) was also shown to react in HCT-8 cells, a human cancer cell line expressing high levels of A-Raf.

### SOURCE:

c-Raf antibody was produced against a chemically synthesized phosphopeptide derived from a region of human c-Raf that contains serine 259. The sequence is conserved in mouse and rat.

c-Raf antibody was purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated c-Raf. The final product is generated by affinity chromatography using a c-Raf-derived peptide that is phosphorylated at serine 259.

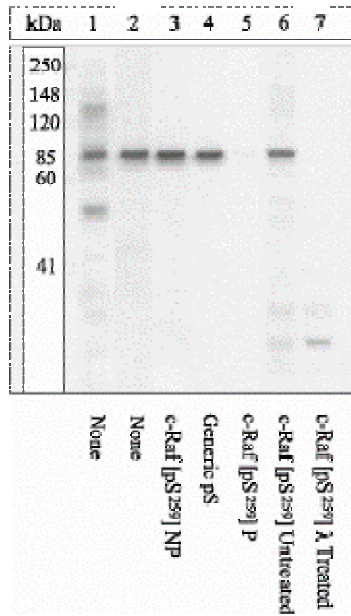
### APPLICATION:

For Western blotting applications, we recommend using the antibody at 0.1-1.0 µg/mL. At 0.50 µg/mL, the dilution provides 100 mL working solution, which at 10 mL/blot allows 10 blots to be performed. . **This product is for research use only.**

### STORAGE:

Store at -80°C. Upon initial thawing, apportion into working aliquots and Store at -80°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.

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Total cell lysates (1) or immunoprecipitates (2-7) prepared from Hek293 cells overexpressing c-Raf and stimulated with EGF were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either untreated (1-6), or treated with lambda (delta) phosphatase (7), blocked with a 5% BSA-TBST buffer overnight at 4°C, then incubated with 0.50 µg/mL c-Raf [pS259] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2, 6, 7), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4), or the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar(TM) method. The data show that only the peptide corresponding to c-Raf [pS259] blocks the antibody signal, thereby demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.