

Product Information

Anti-phospho-c-Raf [pSer⁴³]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 7615**

Product Description

Anti-phospho-c-Raf [pSer⁴³] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human c-Raf that contains serine 43 as immunogen. The sequence is conserved in mouse and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated c-Raf.

The antibody detects human c-Raf. Mouse and rat (100% homologous) c-Raf have not been tested, but are expected to react. It has been used in immunoblotting applications.

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 43 of c-Raf is phosphorylated by PKA, and is thought to negatively affect the ability of Ras to activate c-Raf.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at -70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

One vial is sufficient for 10 immunoblots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using immunoprecipitates of EGF-stimulated Hek293 cells, transfected with c-Raf.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Immunoprecipitates prepared from Hek293 cells over-expressing c-Raf and left unstimulated (Lane 1) or stimulated with EGF (Lanes 2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
Lane 1 & 5 no peptide
Lane 2 immunogen
Lane 3 a generic phosphoserine containing peptide
Lane 4 non phosphorylated peptide corresponding to the immunogen
4. After preincubation membranes were incubated with 0.50 µg/mL c-Raf [pSer⁴³] antibody for two hours at room temperature in a 3% BSA-TBST buffer.

- After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 show that only the peptide corresponding to c-Raf [pSer⁴³] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

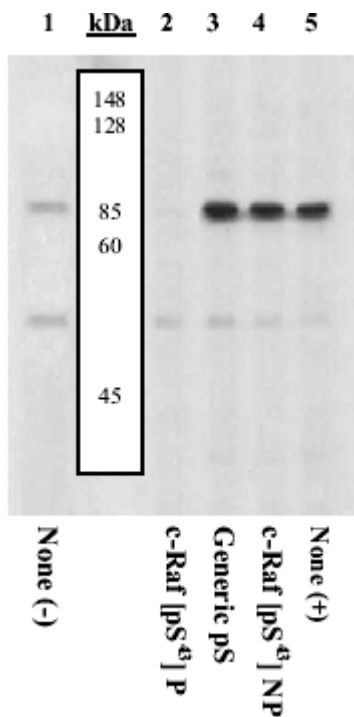


Figure 1 Peptide Competition

References

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