

## Product Information

### Anti-phospho-c-Raf [pSer<sup>338</sup>/pTyr<sup>340</sup>]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 7365**

#### Product Description

Anti-phospho-c-Raf [pSer<sup>338</sup>/pTyr<sup>340</sup>] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human c-Raf that contains serine 338 and tyrosine 340 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 non-phosphorylated c-Raf.

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

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. Phosphorylation of serine 338 creates a recognition site for tyrosine kinases and is critical for the activation of c-Raf. Serine 338 to tyrosine 341 (SSYY) is the phosphoregulatory site which regulates the biological function of the c-Raf tyrosine kinase by oncogenic Ras and Src.

#### Reagent

The antibody is supplied as 100  $\mu$ L of a solution in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 50% glycerol, 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  $-20^{\circ}\text{C}$ . Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

#### Product Profile

One vial is sufficient for 10 immunoblots.

A recommended working dilution of 1:1000 is determined by immunoblotting using immunoprecipitates or cell lysates from Hek293 cells transfected with c-Raf, or co-transfected with c-Raf, Ras[V12] and Rac1.

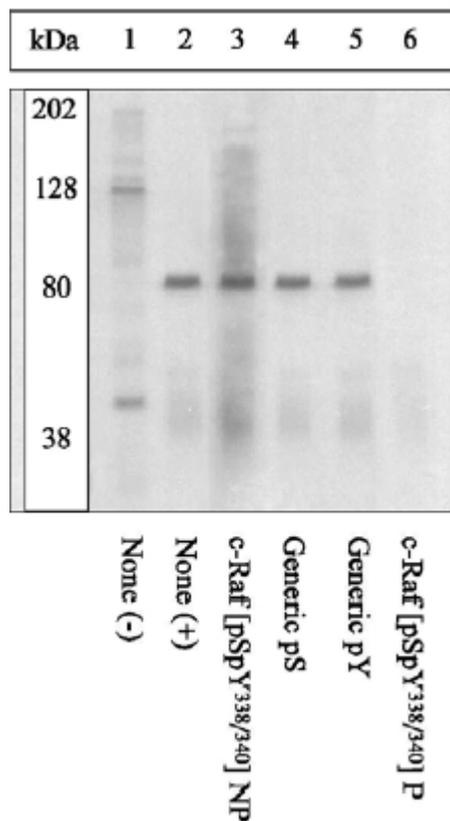
**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### Peptide Competition

- Total cell lysates prepared from Hek293 cells over-expressing c-Raf alone (Lane 1) or immunoprecipitates cotransfected with Rac1 (Lanes 2-6) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
- Membranes were blocked with a 5% BSA-TBST buffer overnight at  $4^{\circ}\text{C}$ .
- After blocking, membranes were preincubated with different peptides as follows:

Lane 1, 2	no peptide
Lane 3	non phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphoserine containing peptide
Lane 5	a generic phosphotyrosine containing peptide
Lane 6	immunogen

- After preincubation membranes were incubated with 1.0  $\mu\text{g/mL}$  c-Raf [pSer<sup>338</sup>/pTyr<sup>340</sup>] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
- After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected.



**Figure 1 Peptide Competition**

The data show that only the peptide corresponding to c-Raf [pSer<sup>338</sup>/pTyr<sup>340</sup>] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

## References

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