

p-Raf-1 (Ser 621)-R: sc-16807-R

BACKGROUND

Raf-1 is a ubiquitously expressed cytoplasmic protein with intrinsic serine/threonine kinase activity. Raf-1, or c-Raf, is the cellular homolog of v-Raf, the product of the transforming gene of the 3611 strain of murine sarcoma virus. The unregulated kinase activity of the v-Raf protein is associated with cellular transformation and mitogenesis. Raf-1 is normally suppressed by its regulatory N-terminal domain. Raf-1 is activated in response to a variety of tyrosine kinase receptors as well as in response to pp60v-Src expression. Specifically, Raf-1 is phosphorylated in the catalytic domain at Ser 338 and, to a lesser extent, Ser 339. This phosphorylation requires the co-activation of PI 3-kinase and the Ras signaling pathway. Raf-1 is also phosphorylated on Tyr 340 and 341, which induces the phosphorylation of MEK. Phosphorylation of Ser 621 is essential for the catalytic activity of Raf-1 and downregulation by c-AMP-dependent protein kinase A (PKA). PKA also phosphorylates Raf-1 on Ser 43 and Ser 259. PKA phosphorylation of Ser 259 inhibits Raf-1 and decreases the phosphorylation necessary for Raf-1 activation at Ser 338.

CHROMOSOMAL LOCATION

Genetic locus: RAF1 (human) mapping to 3p25.2; Raf1 (mouse) mapping to 6 E3.

SOURCE

p-Raf-1 (Ser 621)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing Ser 621 phosphorylated Raf-1 of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-16807 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-Raf-1 (Ser 621)-R is recommended for detection of Ser 621 phosphorylated Raf-1 of mouse, rat, human and zebrafish origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-Raf-1 (Ser 621)-R is also recommended for detection of correspondingly phosphorylated Ser on Raf-1 in additional species, including equine, canine, bovine, porcine and avian.

Suitable for use as control antibody for Raf-1 siRNA (h): sc-29462, Raf-1 siRNA (m): sc-29463, Raf-1 shRNA Plasmid (h): sc-29462-SH, Raf-1 shRNA Plasmid (m): sc-29463-SH, Raf-1 shRNA (h) Lentiviral Particles: sc-29462-V and Raf-1 shRNA (m) Lentiviral Particles: sc-29463-V.

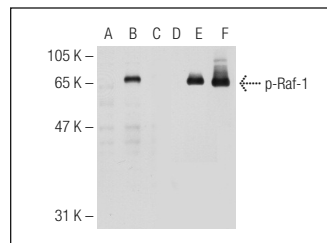
Molecular Weight of p-Raf-1: 74 kDa.

Positive Controls: Raf-1 (m): 293T Lysate: sc-122942, HeLa + UV irradiated cell lysate: sc-2221 or HeLa + PMA/PE whole cell lysate: sc-24808.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



Western blot analysis of Raf-1 phosphorylation in non-transfected: sc-117752 (A, D), untreated mouse Raf-1 transfected: sc-122942 (B, E) and lambda protein phosphatase treated mouse Raf-1 transfected: sc-122942 (C, F) 293T whole cell lysates. Antibodies tested include p-Raf-1 (Ser 621)-R: sc-16807-R (A, B, C) and Raf-1 (540): sc-52827 (D, E, F).

SELECT PRODUCT CITATIONS

1. Noble, C., et al. 2008. CRAF autophosphorylation of Serine 621 is required to prevent its proteasome-mediated degradation. *Mol. Cell* 31: 862-872.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.


 MONOS
Satisfaction
Guaranteed

Try **p-Raf-1 (E-1): sc-271929** or **p-Raf-1 (H-8): sc-271928**, our highly recommended monoclonal alternatives to p-Raf-1 (Ser 621).