SANTA CRUZ BIOTECHNOLOGY, INC.

p-Rad9 (Ser 277): sc-130213

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G_1 to S or G_2 to M phase checkpoints by conserved regulatory mechanisms. Chk1, Rad9 and Hus1 are involved in the signal transduction cascade that regulates cell cycle arrest at the G_2 checkpoint. Chk1 functions as an essential component in the G_2 phase DNA damage checkpoint, as it phosphorylates Cdc25C in response to DNA damage and thereby inhibits mitosis. Two related mammalian proteins, Hus1 and Rad9, share conserved sequence identity and function to the yeast homologs of the same names. *In vivo*, Rad9 is highly phosphorylated and directly associates with two other checkpoint control proteins, Rad1 and Hus1. Additionally, Rad9 associates with anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-x_L, but not with the pro-apoptotic Bax and Bad proteins. Overexpression of Rad9 induces apoptosis and indicates that Rad9 may have an additional role in regulating apoptosis after DNA damage. Human Rad9 may be phosphorylated on specific amino acid residues, including Ser 277.

REFERENCES

1. Carr, A.M., et al. 1995. The Chk1 pathway is required to prevent mitosis following cell-cycle arrest at "start". Curr. Biol. 5: 1179-1190.
2. Lieberman, H.B., et al. 1996. A human homolog of the *Schizosaccharomyces pombe* Rad9+ checkpoint control gene. Proc. Natl. Acad. Sci. USA 93: 13890-13895.
3. Sanchez, Y., et al. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.
4. O'Connell, M.J., et al. 1997. Chk1 is a Wee1 kinase in the G_2 DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. EMBO J. 16: 545-554.
5. Peng, C.Y., et al. 1997. Mitotic and G_2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on Serine 216. Science 277: 1501-1505.
6. Kostrub, C.F., et al. 1998. Hus1p, a conserved fission yeast checkpoint protein, interacts with Rad1p and is phosphorylated in response to DNA damage. EMBO J. 17: 2055-2066.
7. St. Onge, R.P., et al. 1999. The human G_2 checkpoint control protein hRAD9 is a nuclear phosphoprotein that forms complexes with hRAD1 and hHUS1. Mol. Biol. Cell. 10: 1985-1995.
8. Komatsu, K., et al. 2000. Human homologue of *S. pombe* Rad9 interacts with Bcl-2/Bcl-x_L and promotes apoptosis. Nat. Cell. Biol. 2: 1-6.

CHROMOSOMAL LOCATION

Genetic locus: RAD9A (human) mapping to 11q13.1.

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.

SOURCE

p-Rad9 (Ser 277) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 277 of Rad9 of human origin.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml PBS with < 0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

p-Rad9 (Ser 277) is recommended for detection of Ser 277 phosphorylated Rad9 of human 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), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

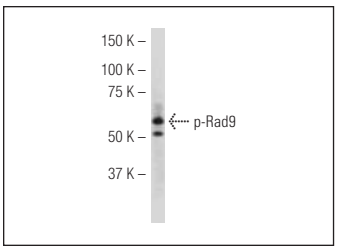
Suitable for use as control antibody for Rad9 siRNA (h): sc-36364, Rad9 shRNA Plasmid (h): sc-36364-SH and Rad9 shRNA (h) Lentiviral Particles: sc-36364-V.

Molecular Weight of Rad9: 65 kDa.

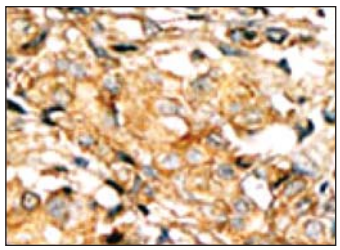
RECOMMENDED SECONDARY REAGENTS

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 A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 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. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



p-Rad9 (Ser 277): sc-130195. Western blot analysis of p-Rad9 expression in Y79 whole cell lysate.



p-Rad9 (Ser 277): sc-130213. Immunoperoxidase staining of formalin fixed, paraffin-embedded human cancer tissue showing cytoplasmic staining.

PROTOCOLS

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