SANTA CRUZ BIOTECHNOLOGY, INC.

Rad9 (A-4): sc-74463

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G_1/S or G_2/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.

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. 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.
5. 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.
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.

CHROMOSOMAL LOCATION

Genetic locus: RAD9A (human) mapping to 11q13.2; Rad9 (mouse) mapping to 19 A.

SOURCE

Rad9 (A-4) is a mouse monoclonal antibody raised against amino acids 1-389 representing full length Rad9 of mouse origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

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

APPLICATIONS

Rad9 (A-4) is recommended for detection of Rad9 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, 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).

Suitable for use as control antibody for Rad9 siRNA (h): sc-36364, Rad9 siRNA (m): sc-36365, Rad9 shRNA Plasmid (h): sc-36364-SH, Rad9 shRNA Plasmid (m): sc-36365-SH, Rad9 shRNA (h) Lentiviral Particles: sc-36364-V and Rad9 shRNA (m) Lentiviral Particles: sc-36365-V.

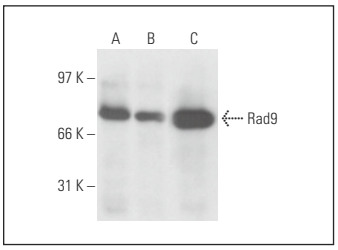
Molecular Weight of Rad9: 65 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, HeLa nuclear extract: sc-2120 or KNRK nuclear extract: sc-2141.

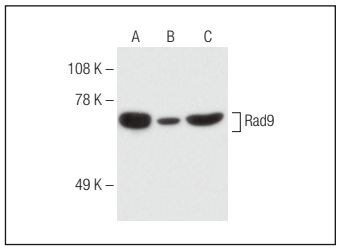
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 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 m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

DATA



Rad9 (A-4): sc-74463. Western blot analysis of Rad9 expression in HeLa whole cell lysate (**A**) and HeLa (**B**) and KNRK (**C**) nuclear extracts.



Rad9 (A-4): sc-74463. Western blot analysis of Rad9 expression in KNRK (**A**), PC-3 (**B**) and CCRF-CEM (**C**) nuclear extracts.

RESEARCH USE

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

PROTOCOLS

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