

Rad9 (B-6): sc-166457

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G₁/S or G₂/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 G₂ checkpoint. Chk1 functions as an essential component in the G₂ phase DNA damage checkpoint, phosphorylating Cdc25C in response to DNA damage and thereby inhibiting 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. O'Connell, M.J., et al. 1997. Chk1 is a Wee 1 kinase in the G₂ DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. *EMBO J.* 16: 545-554.
5. Peng, C.Y., et al. 1997. Mitotic and G₂ 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.

CHROMOSOMAL LOCATION

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

SOURCE

Rad9 (B-6) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 360-390 at the C-terminus of Rad9 of human origin.

PRODUCT

Each vial contains 200 µg IgG₁ kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-166457 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

Rad9 (B-6) 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.

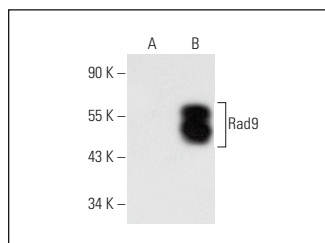
Molecular Weight of Rad9: 65 kDa.

Positive Controls: PC-3 nuclear extract: sc-2152, CCRF-CEM nuclear extract: sc-2146 or Rad9 (h): 293T Lysate: sc-113776.

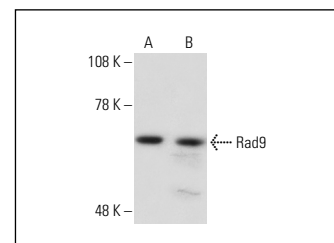
RECOMMENDED SUPPORT REAGENTS

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 (B-6): sc-166457. Western blot analysis of Rad9 expression in non-transfected: sc-117752 (A) and human Rad9 transfected: sc-113776 (B) 293T whole cell lysates.



Rad9 (B-6): sc-74464. Western blot analysis of Rad9 expression in PC-3 (A) and CCRF-CEM (B) nuclear extracts.

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 for detailed protocols and support products.