

Rad9 (h): 293T Lysate: sc-113776

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. Kostub, 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 G₂ 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.
9. Abdu, U., et al. 2007. An essential role for *Drosophila* Hus1 in somatic and meiotic DNA damage responses. *J. Cell Sci.* 120: 1042-1049.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

PROTOCOLS

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

CHROMOSOMAL LOCATION

Genetic locus: RAD9A (human) mapping to 11q13.2.

PRODUCT

Rad9 (h): 293T Lysate represents a lysate of human Rad9 transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

APPLICATIONS

Rad9 (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Rad9 antibodies. Recommended use: 10-20 µl per lane.

Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

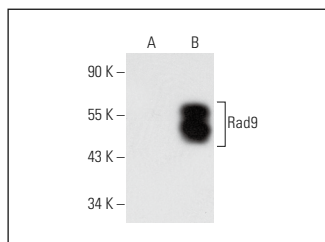
Rad9 (B-6): sc-166457 is recommended as a positive control antibody for Western Blot analysis of enhanced human Rad9 expression in Rad9 transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

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.

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.

RESEARCH USE

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