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## anti-RAD9A antibody (pSer1129)

2 Images



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Quantity:	100 μg	
Target:	RAD9A	
Binding Specificity:	AA 1125-1139, pSer1129	
Reactivity:	Saccharomyces cerevisiae	
Host:	Rabbit	
Clonality:	Polyclonal	
Conjugate:	This RAD9A antibody is un-conjugated	
Application:	ELISA, Immunohistochemistry (IHC)	
Product Details		
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated	
	immunizations with a synthetic peptide corresponding to phosphorylated form of aa 1125-1139	
	of 1309 of yeast Rad9 protein conjugated to KLH.	
Isotype:	IgG	
Characteristics:	Concentration Definition: by UV absorbance at 280 nm	
Target Details		
Target:	RAD9A	
Alternative Name:	Rad9 (RAD9A Products)	
Background:	Rad9 is required for the MEC1/TEL1-dependent activation of Saccharomyces cerevisiae DNA	

damage checkpoint pathways mediated by Rad53 and Chk1. DNA damage induces Rad9

phosphorylation, and Rad53 specifically associates with phosphorylated Rad9. Cells have evolved multiple strategies for tolerating genomic damage. The most important of these are numerous repair systems that remove or bypass potentially mutagenic DNA lesions. Another cellular strategy is to delay cell-cycle transitions at multiple points. The genetic control of these delays, termed 'checkpoints', was first established in budding yeast where it was shown that the RAD9 gene functions in G<sub>2</sub>/M arrest after irradiation with X-rays. Subsequently, it has become clear that Rad9 also functions at the G<sub>1</sub>/S, intra-S and mid-anaphase checkpoints. Defects in checkpoint regulation can lead to genome instability and, in higher eukaryotes, neoplastic transformation. Rad9 also controls the transcriptional induction of a DNA damage regulon (DDR). Rad9 may also have a pro-apoptotic function. This is suggested in that Rad9 from Schizosaccharomyces pombe (SpRad9) contains a group of amino acids with similarity to the Bcl-2 homology 3 death domain, which is required for SpRad9 interaction with human Bcl-2 and apoptosis induction in human cells. Overexpression of Bcl-2 in S. pombe inhibits cell growth independently of rad9, but enhances resistance of rad9-null cells to methyl methanesulfonate, ultraviolet and ionizing radiation. Rad9 conveys the checkpoint signal by activating Rad53p and Chk1p; is hyperphosphorylated by Mec1p and Tel1p; and is a potential Cdc28p substrate. Mature yeast Rad9 is reported to have an apparent molecular weight of ~148kDa. The human homolog is reported at 48.5 kDa. Synonyms: Cell cycle checkpoint control protein antibody, Cell cycle checkpoint control protein RAD9A antibody, DNA repair exonuclease rad9 homolog A antibody

Gene ID:

5883, 4759022

UniProt:

P14737

Pathways:

Positive Regulation of Response to DNA Damage Stimulus

#### **Application Details**

Application Notes:

This phospho specific polyclonal antibody was tested by ELISA. Data from ELISA indicates the antibody is reactive with the phosphorylated form of the immunizing peptide and minimally reactive with the non-phosphorylated form of the immunizing peptide. No reactivity is expected against the human or mouse analogs of RAD9. Reactivity against RAD9 from other sources is unknown. Although not tested, this antibody is likely functional by WB, IHC and IP. This product has been assayed against 0.1 µg of phosphorylated peptide (pS1129) in a standard capture ELISA using TMB (3,3',5,5'-Tetramethylbenizidine) as a substrate for 30 minutes at room temperature. A working dilution of 1:5,000 is suggested for this product. Minimal reactivity was detected against the non-phosphorylated form (S1129) of the immunizing peptide. This

#### **Application Details**

antibody appears to be specific for the active form (phosphorylated) of the protein.
Researchers should determine optimal titers for other applications.

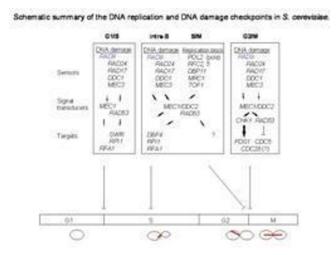
Restrictions:

For Research Use only

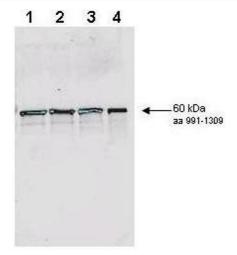
#### Handling

Format:	Liquid	
Concentration:	0.43 mg/mL	
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2	
Preservative:	Sodium azide	
Precaution of Use:	caution of Use: This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.	
Storage:	-20 °C	

### **Images**



**Image 1.** Checkpoints are mechanisms that impose delays in the cell cycle in response to DNA damage or defects in DNA replication, to ensure that mitotic transmission is errorfree. Failure to delay the cell cycle in the presence of damage converts an easily reparable DNA lesion into one far more deleterious, provoking genomic instability or cell death. This figure shows a summary of our current knowledge about the DNA damage checkpoints in yeast. Genetic analysis of the pathway has allowed classification of its components into Sensors, which detect different sorts of damage, Signal transducers which are signal-integrating kinases, and Targets which carry out the essential functions of suppressing progress through the cell cycle (i.e. inducing repair genes and preventing late origin firing or sister chromatid segregation. Contributed by C. Frei and K. Shimada, laboratory of S. Gasser, U. Geneva.



#### **Western Blotting**

Image 2. Affinity purified antibody to yeast Rad9 (1125-1139 pan reactive) was used at a 1:200 dilution incubated 8 h at room temperature to detect Rad9 by Western blot. Lanes were loaded with 50 ng each of recombinant GST fusion protein containing S. cerevisiae Rad9 (aa 991-1309 ~60 kDa) on a 4-20% Criterion gel for SDS-PAGE as follows: Lane 1 - non-phosphorylated wild type yeast Rad9, Lane 2 in vitro phosphorylated wild type yeast Rad9, Lane 3 - nonphosphorylated S1129A/S1260A double mutant Rad9, Lane 4 - in vitro phosphorylated S1129A/S1260A double mutant. Phosphorylation of Rad9 was by treatment with ATP and Rad53 kinase. Detection occurred using a 1:5,000 dilution of800 conjugated Donkey anti-Rabbit IgG (code # 611-732-127) for 1h at room temperature. LICOR's Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.