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Datasheet for 600-401-468

Rad9 phospho S1260 Antibody

Overview

Description:	Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - 600-401-468
Item No.:	600-401-468
Size:	100 µg
Applications:	ELISA, WB
Reactivity:	Yeast
Host Species:	Rabbit

Product Details

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 G2/M arrest after irradiation with X-rays. Subsequently, it has become clear that Rad9 also functions at the G1/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 hyperphos-phorylated 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:	Rabbit anti-Rad9 pS1260 antibody, Rabbit anti-Rad9 phospho antibody, Rad 9, Rad-9, Cell cycle checkpoint control protein antibody, Cell cycle checkpoint control protein RAD9A antibody, DNA repair exonuclease rad9 homolog A antibody, DNA repair protein RAD9, YDR217C, YD9934.02C
Host Species:	Rabbit

Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	RAD9
Reactivity:	Yeast
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to phosphorylated form of an internal region near aa 1225-1275 from the aa1309 yeast Rad9 protein conjugated to KLH.
Purity/Specificity:	This affinity purified antibody is directed against the phosphorylated form of yeast Rad9 at the pS1260 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody is phospho specific to yeast pS1260.
Relevant Links:	• NCBI - NP_010503.1 • UniProtKB - P14737 • GeneID - 851803

Application Details

Tested Applications:	ELISA, WB
Application Note:	This phospho specific polyclonal antibody was tested by immunoblotting and ELISA. Data from both immunoblotting and ELISA indicate the antibody is reactive with the phosphorylated form of the immunizing peptide and minimally reactive with the non-phosphorylated form of the immunizing peptide. Immunoblotting detects yeast Rad9 protein. No reactivity is expected against the human or mouse analogs of RAD9. Reactivity against RAD9 from other sources is unknown. Cross reactivity may occur with auto-phosphorylated Rad53 kinase. Although not tested, this antibody is likely functional by IHC and IP. This product has been assayed against 0.1 µg of phosphorylated peptide (pS1260) in a standard capture ELISA using TMB (3,3',5,5'-Tetramethylbenizidine) code # TMBE-100 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 (S1260) of the immunizing peptide. This antibody appears to be specific for the active form (phosphorylated) of the protein. Dilute the antibody 1:100 to 1:500 for immunoblotting. Researchers should determine optimal titers for other applications.

Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000
WB:	1:500- 1:2,000

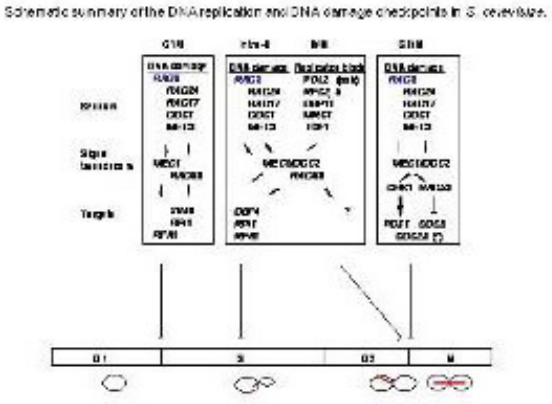
Formulation

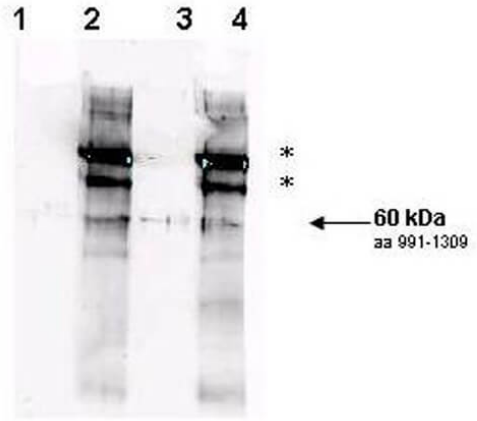
Physical State:	Liquid (sterile filtered)
Concentration:	0.41 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images





Pathway

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 error-free. 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 Blot

Affinity purified phospho-specific antibody to yeast Rad9 at pS1260 was used at a 1:200 dilution incubated overnight at 4° C 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 - non-phosphorylated 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. Rad53 kinase autophosphorylates and appears cross reactive as it is detected on the blot as 90 and 110 kDa bands (asterisk). Detection occurred using a 1:5,000 dilution of IRDye™800 conjugated Donkey anti-Rabbit IgG (code # 611-732-127) for 1h at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.

Disclaimer

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