

**Anti-Yeast Rad9 pS1260 (RABBIT) Antibody**  
**Rad9 phospho S1260 Antibody**  
**Catalog # ASR5244****Specification**

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**Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - Product Information**

Host	Rabbit
Conjugate	Unconjugated
Target Species	Yeast
Reactivity	Yeast
Clonality	Polyclonal
Application	WB, E, I, LCI
Application Note	<p>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'-Tetramethylbenzidine) 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.</p>
Physical State	Liquid (sterile filtered)
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Immunogen	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to

Preservative

phosphorylated form of an internal region  
near aa 1225-1275 from the aa1309 yeast  
Rad9 protein conjugated to KLH.  
0.01% (w/v) Sodium Azide

### Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - Additional Information

Gene ID 851803

Other Names  
851803

#### Purity

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.

#### 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.

#### Precautions Note

This product is for research use only and is not intended for therapeutic or diagnostic applications.

### Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - Protein Information

Name RAD9

#### Function

Essential for cell cycle arrest at the G2 stage following DNA damage by X-irradiation or inactivation of DNA ligase.

#### Cellular Location

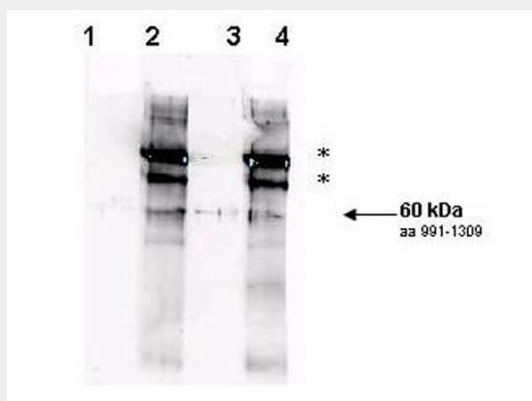
Nucleus.

### Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

### Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - Images



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.

#### **Anti-Yeast Rad9 pS1260 (RABBIT) Antibody - 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 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.