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Cell Cycle related Antibodies for Epigenetics Research

Anti-Polymerase eta coded by RAD30 gene from Saccharomyces cerevisiae Cat # RAD-101AP

Saccharomyces cerevisiae Radiation sensitive protein 30 (Rad30) is the homolog of human DNA polymerase eta. The Saccharomyces cerevisiae RAD30 gene, a homologue of Escherichia coli dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism. Both human and yeast polymerase eta are responsible for error-free bypass of UV-induced cis-syn pyrimidine dimers and several other DNA lesions. The RAD30A gene encodes DNA polymerase eta and defects in the protein lead to the xeroderma pigmentosum variant (XP-V) phenotype in humans. The hypermutations in all 4 bases in immunoglobulin genes are the result of low fidelity DNA polymerases like Poleta encoded by Rad30 in S. cerevisiae, RAD 30 is post-translationally modified by Ubiquitination and proteasome mediated degradation. The biological half life for Rad30 is 20 minutes and is increased due to proteasome defects (1). Other mutation inactivating proteins like Skp and F-box protein Ufo1 stabilize Rad30. UV radiation causes transient stabilization of Rad30 which tend to accumulate this protein in the cells. DNA polymerase eta, involved in the predominantly error-free bypass replication of DNA lesions, catalyzes the efficient and accurate synthesis of DNA opposite cyclobutane pyrimidine dimers; homolog of human POLH and bacterial DinB proteins.

The Rad30 gene resides on S cerevisiae chromosome 4. There are two variants of Rad30 proteins, Rad30-101AP antibodies were generated against 632 amino acid protein from saccharomyces cerevisiae. Rad30 contain several functional domain, including UmuC (26-309); Pol30 binding domain (625-632) and required Mg for its activity. Mutations at Aspartic acid 30 and or at 155, to Alanine abolished the DNA polymerase activity and several other mutations alter the enzymatic activity in different fashion.

The Anti-RAD30 selective antibodies were generated against conserved sequences from amino acid 284-300 of the Rad30 protein. The Rad30-selective antibodies are affinity purified against immobilized antigen based affinity chromatography which yielded high affinity epitope-specific antibodies. The Rad30 antibodies label a protein of approximately 76-78kDa protein in Western blot using Western blot positive control for Rad30 (PC-Rad30). Anti-Rad30-selective antibodies are also available in affinity-purified form for confocal, Western blotting and immunocytochemical analyses. FabGennix Inc. will also conjugate antibodies with fluorescent probes upon request at extra charge. Limited quantities of antigenic peptide is also available (inquire before ordering). FabGennix Inc. also provides antibodies to other antibodies for epigenetics research, for a complete listing please visit www.FabGennix.com. FabGennix Int. Inc., will also provide Western blot positive controls for most of these antibodies in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigens are also available for blocking studies. Please inquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity
Rad30-101AP	Rabbit	Affinity purified Rad30 Antibody	Saccharomyces cerevisiae	150-175°l
PC-Rad30	n/a	Western blot positive control for Rad30	n/a	5 appl
P-Rad30	n/a	Antigenic blocking peptide for Rad30-101	n/a	250 °g

R = rat; M = mouse; H = human; C = chicken; monk = monkey; Sh = sheep; B = bovine ; * not all variants are labeled equally

Immunogen: Synthetic peptide, amidated peptide for Rad30, the peptide was cyclized by creating disulfide bonds between two cys. The peptide was conjugated to KLH & is used to generate antibodies in rabbit.

Concentration: Rad30-101AP 0.70-0.98 mg/ml of antibody stabilization buffer

Applications: Rad30-101AP is tested for WB application at 1:500 dilution. Other applications for this antibody has not been tested. WB: > 1:500; IMM & i.p pull-down assays: n.d; IHC n.d (Antibody dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions).

Protocols: Standard protocol for various applications (WB, IMM, IHC) of this antibody can be obtained upon request. The specification sheet for Rad30 antibody will be supplied with each product. FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% azide. The purified antibodies are isolated from immobilized antigen affinity column. The affinity pure antibodies are supplied as 0.5-1 mg/ml IgG in antibody stabilization buffer. For long-term storage of keep at -20°C. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µ filter after every use for long-term storage.

Notes: Now Western blots can easily be stripped and recycle using our specially formulated StripOBuffer (Cat # FGI-1989). This stripping buffer does not require heating or have any pungent smell.

References:

1. Skoneczna A, McIntyre J, Skoneczny M, Policinska Z, Sledziewska-Gojska E. Polymerase eta is a short-lived, proteasomally degraded protein that is temporarily stabilized following UV irradiation in Saccharomyces cerevisiae. J Mol Biol. 2007 Mar 2;366(4):1074-86. Epub 2006 Dec 6.
2. Parker JL, Bielen AB, Dikic I, Ulrich HD. Contributions of ubiquitin- and PCNA-binding domains to the activity of Polymerase eta in Saccharomyces cerevisiae. Nucleic Acids Res. 2007;35(3):881-9. Epub 2007 Jan 23.

* For users who may require large amounts of Rad30-101AP, please enquire about bulk material discounts.

This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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