Anti-Rad21 antibody - ChIP Grade ab992

Overview

Product name: Anti-Rad21 antibody - ChIP Grade
Description: Rabbit polyclonal to Rad21 - ChIP Grade
Host species: Rabbit
Specificity: The epitope recognized by ab992 maps to a region between residue 575 and the C-terminus (residue 631) human Rad21 homolog using the numbering given in entry NP_006256.1 (GeneID 5885).

Tested applications: Suitable for: IHC-P, ChIP, ICC/IF, WB, IP
Species reactivity: Reacts with: Mouse, Human, Xenopus laevis, Indian muntjac
Predicted to work with: Rat, Rabbit, Horse, Chicken, Guinea pig, Cow, Dog, Turkey, Chimpanzee, Gorilla, Chinese hamster, Orangutan, Elephant

Immunogen: Synthetic peptide (Human) conjugated to KLH - which represented a portion of human Rad21 encoded within exon 14 (LocusLink ID 5885).
Positive control: HeLa cell lysate. FFPE human breast fibroadenoma tissue sections.

Properties

Form: Liquid
Storage buffer: Preservative: 0.1% Sodium azide
Constituents: 0.021% PBS, 1.764% Sodium citrate, 1.815% Tris
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab992 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
Cleavable component of the cohesin complex, involved in chromosome cohesion during cell cycle, in DNA repair, and in apoptosis. The cohesin complex is required for the cohesion of sister chromatids after DNA replication. The cohesin complex apparently forms a large proteinaceous ring within which sister chromatids can be trapped. At metaphase-anaphase transition, this protein is cleaved by separase/ESPL1 and dissociates from chromatin, allowing sister chromatids to segregate. The cohesin complex may also play a role in spindle pole assembly during mitosis. Also plays a role in apoptosis, via its cleavage by caspase-3/CASP3 or caspase-7/CASP7 during early steps of apoptosis: the C-terminal 64 kDa cleavage product may act as a nuclear signal to initiate cytoplasmic events involved in the apoptotic pathway.

Sequence similarities
Belongs to the rad21 family.

Domain
The C-terminal part associates with the head of SMC1A, while the N-terminal part binds to the head of SMC3.

Post-translational modifications
Cleaved by separase/ESPL1 at the onset of anaphase. Cleaved by caspase-3 and caspase-7 at the beginning of apoptosis. The cleavage by ESPL1 and caspase-3 take place at different sites.
Phosphorylated; becomes hyperphosphorylated in M phase of cell cycle. The large dissociation of cohesin from chromosome arms during prophase may be partly due to its phosphorylation by PLK.

Cellular localization
Nucleus. Chromosome. Chromosome > centromere. Associates with chromatin. Before prophase it is scattered along chromosome arms. During prophase, most of cohesin complexes dissociate from chromatin probably because of phosphorylation by PLK, except at centromeres, where cohesin complexes remain. At anaphase, it is cleaved by separase/ESPL1, leading to the dissociation of the complex from chromosomes, allowing chromosome separation. Once cleaved by caspase-3, the C-terminal 64 kDa cleavage product translocates to the cytoplasm, where it may trigger apoptosis.

Application
<table>
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<tr>
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<th>Abreviews</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>ChiP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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| ICC/IF      | ⭐⭐⭐⭐⭐    | Use at an assay dependent concentration.  
Used at a dilution of 1/200 for 30 min incubation (see Abreview for further information). |
Band observed at ~130 kDa. |
| IP          | ⭐⭐⭐⭐⭐    | Use a concentration of 1 - 4 µg/ml. |

Target

Images

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Western blot - Anti-Rad21 antibody - ChIP Grade (ab992) at 1/1000 dilution + HeLa whole cell lysate

Developed using the ECL technique.

**Predicted band size:** 72 kDa  
**Observed band size:** 75 kDa  
**Additional bands at:** 130 kDa (possible cross reactivity)

Immunocytochemistry/ Immunofluorescence - Anti-Rad21 antibody - ChIP Grade (ab992) at a 1/200 dilution staining

Asynchronous and paraformaldehyde-fixed (4%) HeLa cells by immunocytochemistry. The antibody was incubated with the cells 30 minutes and then detected using a Cy3 conjugated Goat Anti-Mouse IgG (H+L) antibody.

This image is courtesy of an Abview by Kirk McManus submitted on 27 February 2006.
IHC image of Rad21 staining in human breast fibroadenoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab992, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Anti-Rad21 antibody - ChIP Grade (ab992) at 1/2000 dilution + HeLa whole cell extract (extraction was achieved using RIPA buffer and 1% SDS) at 25 µg/ml

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/1500 dilution

Predicted band size: 72 kDa

A rad21 knock down negative control was employed.

This image is courtesy of an Abreview submitted on 9 September 2005. We do not have any further information relating to this image.
All lanes: Anti-Rad21 antibody - ChIP Grade (ab992) at 2 µg/ml

Lane 1: HeLa at 50 µg
Lane 2: HeLa at 15 µg
Lane 3: HeLa at 5 µg
Lane 4: 293T at 50 µg
Lane 5: NIH3T3 at 50 µg

Predicted band size: 72 kDa

Exposure time: 30 seconds

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