abcam

Product datasheet

Anti-Rad9 antibody [93A535] ab13600

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Overview

Product name	Anti-Rad9 antibody [93A535]
Description	Mouse monoclonal [93A535] to Rad9
Host species	Mouse
Tested applications	**Suitable for:** IHC-P, ICC/IF, ICC, WB, IP, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Fusion full length protein (Human).
Positive control	This antibody gave a positive result when used in the following methanol fixed cell lines: MCF-7 IHC-P: FFPE human lung tissue sections.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	93A535
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab13600** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

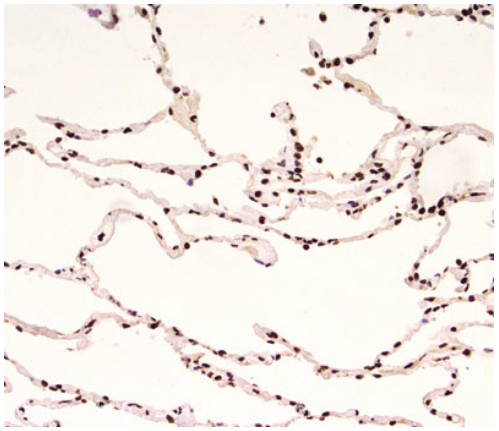
Application	Abreviews	Notes
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Application	Abreviews	Notes
ICC/IF		Use a concentration of 10 µg/ml.
ICC		Use at an assay dependent concentration.
WB		Use a concentration of 1 - 2 µg/ml. Detects a band of approximately 50 kDa (predicted molecular weight: 45 kDa).
IP		Use at an assay dependent concentration.
Flow Cyt		Use 0.01µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function	Component of the 9-1-1 cell-cycle checkpoint response complex that plays a major role in DNA repair. The 9-1-1 complex is recruited to DNA lesion upon damage by the RAD17-replication factor C (RFC) clamp loader complex. Acts then as a sliding clamp platform on DNA for several proteins involved in long-patch base excision repair (LP-BER). The 9-1-1 complex stimulates DNA polymerase beta (POLB) activity by increasing its affinity for the 3'-OH end of the primer-template and stabilizes POLB to those sites where LP-BER proceeds; endonuclease FEN1 cleavage activity on substrates with double, nick, or gap flaps of distinct sequences and lengths; and DNA ligase I (LIG1) on long-patch base excision repair substrates. RAD9A possesses 3'->5' double stranded DNA exonuclease activity. Its phosphorylation by PRKCD may be required for the formation of the 9-1-1 complex.
Sequence similarities	Belongs to the rad9 family.
Post-translational modifications	Constitutively phosphorylated on serine and threonine amino acids in absence of DNA damage. Hyperphosphorylated by PRKCD and ABL1 upon DNA damage. Its phosphorylation by PRKCD may be required for the formation of the 9-1-1 complex.
Cellular localization	Nucleus.

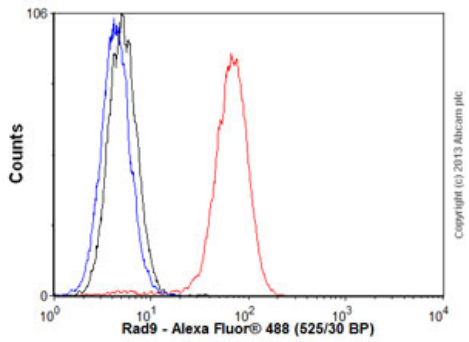
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rad9 antibody [93A535] (ab13600)

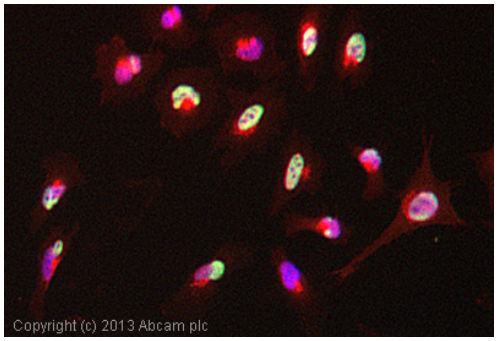
IHC image of Rad9 staining in human lung formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab13600, 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.



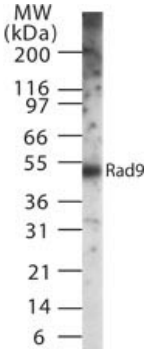
Flow Cytometry - Anti-Rad9 antibody [93A535] (ab13600)

Overlay histogram showing HeLa cells stained with ab13600 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab13600, 0.01µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-Rad9 antibody [93A535] (ab13600)

ICC/IF image of ab13600 stained MCF-7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab13600 at 10µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-Rad9 antibody [93A535] (ab13600)

Western blot analysis of Rad9 using ab13600 at 2 ug/ml against recombinant Rad9.

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