

**RNF8 Antibody**  
**Catalog # ASC11134****Specification**

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**RNF8 Antibody - Product Information**

Application	WB, IF, E
Primary Accession	<a href="#">O76064</a>
Other Accession	<a href="#">NP_003949</a> , <a href="#">4504867</a>
Reactivity	Human, Mouse, Rat
Host	Rabbit
Clonality	Polyclonal
Isotype	IgG
Calculated MW	Predicted: 46, 53 kDa

Application Notes	<b>Observed: 50 kDa KDa</b> RNF8 antibody can be used for detection of RNF8 by Western blot at 1 - 2 µg/mL. Antibody can also be used for immunofluorescence starting at 20 µg/mL. For immunofluorescence start at 20 µg/mL.
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**RNF8 Antibody - Additional Information**

Gene ID **9025**

**Target/Specificity**

RNF8; At least three isoforms of RNF8 are known to exist; this antibody will detect all three isoforms.

**Reconstitution & Storage**

Antibody can be stored at 4°C up to one year. Antibodies should not be exposed to prolonged high temperatures.

**Precautions**

RNF8 Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

**RNF8 Antibody - Protein Information**

**Name** RNF8 {ECO:0000255|HAMAP-Rule:MF\_03067}

**Synonyms** KIAA0646

**Function**

E3 ubiquitin-protein ligase that plays a key role in DNA damage signaling via 2 distinct roles: by mediating the 'Lys-63'-linked ubiquitination of histones H2A and H2AX and promoting the recruitment of DNA repair proteins at double-strand breaks (DSBs) sites, and by catalyzing 'Lys-48'-linked ubiquitination to remove target proteins from DNA damage sites. Following DNA DSBs, it is recruited to the sites of damage by ATM-phosphorylated MDC1 and catalyzes the 'Lys-63'-linked ubiquitination of histones H2A and H2AX, thereby promoting the formation of

TP53BP1 and BRCA1 ionizing radiation-induced foci (IRIF) (PubMed:<a href="http://www.uniprot.org/citations/18001824" target="\_blank">18001824</a>, PubMed:<a href="http://www.uniprot.org/citations/18006705" target="\_blank">18006705</a>). Also controls the recruitment of UIMC1-BRCC3 (RAP80-BRCC36) and PAXIP1/PTIP to DNA damage sites (PubMed:<a href="http://www.uniprot.org/citations/18077395" target="\_blank">18077395</a>, PubMed:<a href="http://www.uniprot.org/citations/19202061" target="\_blank">19202061</a>). Promotes the recruitment of NBN to DNA damage sites by catalyzing 'Lys-6'-linked ubiquitination of NBN (PubMed:<a href="http://www.uniprot.org/citations/23115235" target="\_blank">23115235</a>). Also recruited at DNA interstrand cross-links (ICLs) sites and catalyzes 'Lys-63'-linked ubiquitination of histones H2A and H2AX, leading to recruitment of FAAP20/C1orf86 and Fanconi anemia (FA) complex, followed by interstrand cross-link repair. H2A ubiquitination also mediates the ATM-dependent transcriptional silencing at regions flanking DSBs in cis, a mechanism to avoid collision between transcription and repair intermediates. Promotes the formation of 'Lys- 63'-linked polyubiquitin chains via interactions with the specific ubiquitin-conjugating UBE2N/UBC13 and ubiquitinates non-histone substrates such as PCNA. Substrates that are polyubiquitinated at 'Lys- 63' are usually not targeted for degradation. Also catalyzes the formation of 'Lys-48'-linked polyubiquitin chains via interaction with the ubiquitin-conjugating UBE2L6/UBCH8, leading to degradation of substrate proteins such as CHEK2, JMJD2A/KDM4A and KU80/XRCC5: it is still unclear how the preference toward 'Lys-48'- versus 'Lys-63'- linked ubiquitination is regulated but it could be due to RNF8 ability to interact with specific E2 specific ligases. For instance, interaction with phosphorylated HERC2 promotes the association between RNF8 and UBE2N/UBC13 and favors the specific formation of 'Lys-63'- linked ubiquitin chains. Promotes non-homologous end joining (NHEJ) by promoting the 'Lys-48'-linked ubiquitination and degradation of KU80/XRCC5. Following DNA damage, mediates the ubiquitination and degradation of JMJD2A/KDM4A in collaboration with RNF168, leading to unmask H4K20me2 mark and promote the recruitment of TP53BP1 at DNA damage sites (PubMed:<a href="http://www.uniprot.org/citations/11322894" target="\_blank">11322894</a>, PubMed:<a href="http://www.uniprot.org/citations/14981089" target="\_blank">14981089</a>, PubMed:<a href="http://www.uniprot.org/citations/17724460" target="\_blank">17724460</a>, PubMed:<a href="http://www.uniprot.org/citations/18001825" target="\_blank">18001825</a>, PubMed:<a href="http://www.uniprot.org/citations/18337245" target="\_blank">18337245</a>, PubMed:<a href="http://www.uniprot.org/citations/18948756" target="\_blank">18948756</a>, PubMed:<a href="http://www.uniprot.org/citations/19015238" target="\_blank">19015238</a>, PubMed:<a href="http://www.uniprot.org/citations/19124460" target="\_blank">19124460</a>, PubMed:<a href="http://www.uniprot.org/citations/19203578" target="\_blank">19203578</a>, PubMed:<a href="http://www.uniprot.org/citations/19203579" target="\_blank">19203579</a>, PubMed:<a href="http://www.uniprot.org/citations/20550933" target="\_blank">20550933</a>, PubMed:<a href="http://www.uniprot.org/citations/21558560" target="\_blank">21558560</a>, PubMed:<a href="http://www.uniprot.org/citations/21857671" target="\_blank">21857671</a>, PubMed:<a href="http://www.uniprot.org/citations/21911360" target="\_blank">21911360</a>, PubMed:<a href="http://www.uniprot.org/citations/22266820" target="\_blank">22266820</a>, PubMed:<a href="http://www.uniprot.org/citations/22373579" target="\_blank">22373579</a>, PubMed:<a href="http://www.uniprot.org/citations/22531782" target="\_blank">22531782</a>, PubMed:<a href="http://www.uniprot.org/citations/22705371" target="\_blank">22705371</a>, PubMed:<a href="http://www.uniprot.org/citations/22980979" target="\_blank">22980979</a>). Following DNA damage, mediates the ubiquitination and degradation of POLD4/p12, a subunit of DNA polymerase delta. In the absence of POLD4, DNA polymerase delta complex exhibits higher proofreading activity (PubMed:<a href="http://www.uniprot.org/citations/23233665" target="\_blank">23233665</a>). In addition to its function in damage signaling, also plays a role in higher-order chromatin structure by mediating extensive chromatin decondensation. Involved in the activation of ATM by promoting histone H2B ubiquitination, which indirectly triggers histone H4 'Lys-16' acetylation (H4K16ac), establishing a chromatin environment that promotes efficient activation of ATM kinase. Required in the testis, where it plays a role in the replacement of histones during spermatogenesis. At uncapped telomeres, promotes the joining of deprotected chromosome ends by inducing H2A ubiquitination and TP53BP1 recruitment, suggesting that it may enhance cancer development by aggravating telomere-induced genome instability in case of telomeric crisis. Promotes the assembly of RAD51 at DNA DSBs in the absence of BRCA1 and

TP53BP1 Also involved in class switch recombination in immune system, via its role in regulation of DSBs repair (PubMed:<a href="http://www.uniprot.org/citations/22865450" target="\_blank">22865450</a>). May be required for proper exit from mitosis after spindle checkpoint activation and may regulate cytokinesis. May play a role in the regulation of RXRA-mediated transcriptional activity. Not involved in RXRA ubiquitination by UBE2E2 (PubMed:<a href="http://www.uniprot.org/citations/11322894" target="\_blank">11322894</a>, PubMed:<a href="http://www.uniprot.org/citations/14981089" target="\_blank">14981089</a>, PubMed:<a href="http://www.uniprot.org/citations/17724460" target="\_blank">17724460</a>, PubMed:<a href="http://www.uniprot.org/citations/18001825" target="\_blank">18001825</a>, PubMed:<a href="http://www.uniprot.org/citations/18337245" target="\_blank">18337245</a>, PubMed:<a href="http://www.uniprot.org/citations/18948756" target="\_blank">18948756</a>, PubMed:<a href="http://www.uniprot.org/citations/19015238" target="\_blank">19015238</a>, PubMed:<a href="http://www.uniprot.org/citations/19124460" target="\_blank">19124460</a>, PubMed:<a href="http://www.uniprot.org/citations/19203578" target="\_blank">19203578</a>, PubMed:<a href="http://www.uniprot.org/citations/19203579" target="\_blank">19203579</a>, PubMed:<a href="http://www.uniprot.org/citations/20550933" target="\_blank">20550933</a>, PubMed:<a href="http://www.uniprot.org/citations/21558560" target="\_blank">21558560</a>, PubMed:<a href="http://www.uniprot.org/citations/21857671" target="\_blank">21857671</a>, PubMed:<a href="http://www.uniprot.org/citations/21911360" target="\_blank">21911360</a>, PubMed:<a href="http://www.uniprot.org/citations/22266820" target="\_blank">22266820</a>, PubMed:<a href="http://www.uniprot.org/citations/22373579" target="\_blank">22373579</a>, PubMed:<a href="http://www.uniprot.org/citations/22531782" target="\_blank">22531782</a>, PubMed:<a href="http://www.uniprot.org/citations/22705371" target="\_blank">22705371</a>, PubMed:<a href="http://www.uniprot.org/citations/22980979" target="\_blank">22980979</a>).

### Cellular Location

Nucleus {ECO:0000255|HAMAP-Rule:MF\_03067, ECO:0000269|PubMed:11322894, ECO:0000269|PubMed:14981089, ECO:0000269|PubMed:16215985, ECO:0000269|PubMed:23233665}. Cytoplasm {ECO:0000255|HAMAP-Rule:MF\_03067}. Midbody {ECO:0000255|HAMAP-Rule:MF\_03067}. Chromosome, telomere {ECO:0000255|HAMAP-Rule:MF\_03067} Note=Recruited to uncapped telomeres (By similarity). Following DNA damage, such as double-strand breaks, recruited to the sites of damage (PubMed:18001824, PubMed:18077395, PubMed:22266820, PubMed:23233665) During prophase, concomitant with nuclear envelope breakdown, localizes throughout the cell, with a dotted pattern. In telophase, again in the nucleus and also with a discrete dotted pattern in the cytoplasm. In late telophase and during cytokinesis, localizes in the midbody of the tubulin bridge joining the daughter cells. Does not seem to be associated with condensed chromosomes at any time during the cell cycle. During spermatogenesis, sequestered in the cytoplasm by PIWIL1: RNF8 is released following ubiquitination and degradation of PIWIL1 {ECO:0000255|HAMAP-Rule:MF\_03067, ECO:0000269|PubMed:18001824, ECO:0000269|PubMed:18077395, ECO:0000269|PubMed:22266820, ECO:0000269|PubMed:23233665}

### Tissue Location

Ubiquitous. In fetal tissues, highest expression in brain, thymus and liver. In adult tissues, highest levels in brain and testis, lowest levels in peripheral blood cells

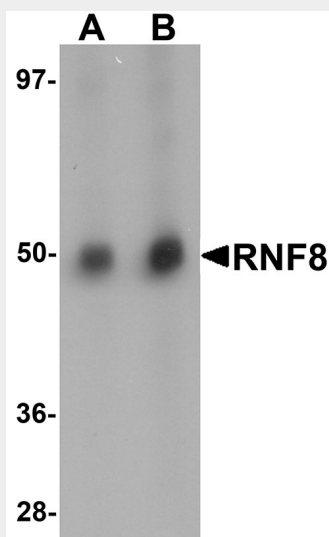
### RNF8 Antibody - Protocols

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

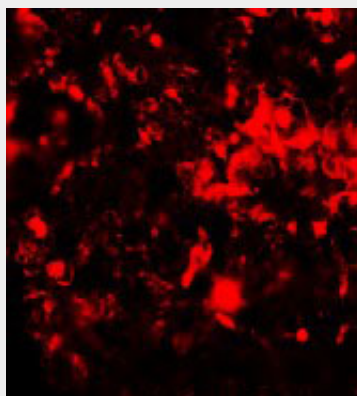
- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)

- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

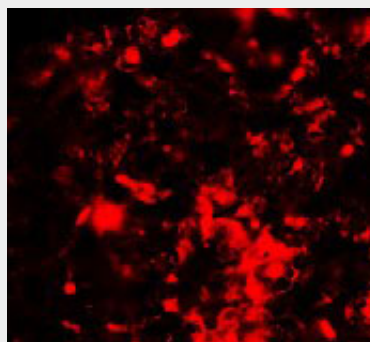
#### RNF8 Antibody - Images



Western blot analysis of RNF8 in human lung tissue lysate with RNF8 antibody at (A) 1 and (B) 2  $\mu\text{g/mL}$ .



Immunofluorescence of RNF8 in rat lung tissue with RNF8 antibody at 20  $\mu\text{g/mL}$ .



Immunofluorescence of RNF8 in Rat Lung tissue with RNF8 antibody at 20  $\mu\text{g/mL}$ .

#### RNF8 Antibody - Background

**RNF8 Antibody:** RNF8 was identified as a ubiquitin ligase (E3) containing a RING finger motif and a FHA domain. This protein has been shown to interact with several class II ubiquitin-conjugating enzymes including UBE2E1/UBCH6, UBE2E2, and UBE2E3. RNF8 assembles at DNA double-strand breaks (DSBs) via interactions through the FHA domain with the adaptor protein MDC1, resulting in an increase in DSB-associated H2A histone ubiquitinations mediated by the associated ubiquitin ligase RNF168 followed by the accumulation of 53BP1 and BRCA1 repair proteins. Together with RNF168, RNF8 plays an integral part of class switch recombination in B cells, allowing the production of several classes of antibodies, through the recruitment of 53BP1 and BRCA1 to the DSB sites.

#### **RNF8 Antibody - References**

Ito K, Adachi S, Iwakami R, et al. N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. *Eur. J. Biochem.* 2001; 268:2725-32.

Mailand N, Bekker-Jensen S, Fastrup H, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007; 131:887-900.

Doil C, Mailand N, Bekker-Jensen S, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 2009; 136:435-46.

Ramachandran S, Chahwen R, Nepal RN, et al. The RNF8/RNF168 ubiquitin ligase cascade facilitates class switch recombination. *Proc. Natl. Acad. Sci. USA* 2010; 107:809-14.