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Product datasheet

Anti-p95/NBS1 antibody [7E4C2] ab181780

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9 Images

Overview

Product name	Anti-p95/NBS1 antibody [7E4C2]
Description	Mouse monoclonal [7E4C2] to p95/NBS1
Host species	Mouse
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human p95/NBS1 aa 467-615. Expressed in *E. Coli*. Sequence: ERDEENQEMSSCKSARIETSCSLLEQTQPATPSLWKNKE QHLSENEPVDT NSDNNLFTDTDLKSIVKNSASKSHAAEKLRSNKKREMDD VAIEDEVLEQL FKDTKPELEIDVKVQKQEEDVNVRKRPRMDIETNDTFSDE AVPESSKIS Database link: **O60934** **Run BLAST with** **Run BLAST with**
Positive control	Human p95/NBS1 recombinant protein; HEK293 cell lysate, transfected with p95/NBS1 (amino acids 467-615)-IgGFc; Jurkat cell lysate; HeLa cells; Human cervical cancer and colon cancer tissues. WB: A431 cell lysate.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: 99% PBS
Purity	Protein G purified
Purification notes	Purified from tissue culture supernatant.
Clonality	Monoclonal
Clone number	7E4C2
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab181780 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
WB		1/500 - 1/2000. Predicted molecular weight: 85 kDa.
ICC/IF		1/200 - 1/1000.
Flow Cyt		1/200 - 1/400. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
IHC-P		1/200 - 1/1000.

Target

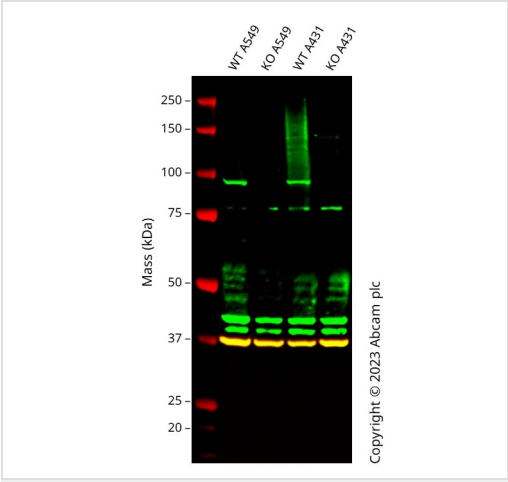
Function Component of the MRE11-RAD50-NBN (MRN complex) which plays a critical role in the cellular response to DNA damage and the maintenance of chromosome integrity. The complex is involved in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity, cell cycle checkpoint control and meiosis. The complex possesses single-strand endonuclease activity and double-strand-specific 3'-5' exonuclease activity, which are provided by MRE11A. RAD50 may be required to bind DNA ends and hold them in close proximity. NBN modulate the DNA damage signal sensing by recruiting PI3/PI4-kinase family members ATM, ATR, and probably DNA-PKcs to the DNA damage sites and activating their functions. It can also recruit MRE11 and RAD50 to the proximity of DSBs by an interaction with the histone H2AX. NBN also functions in telomere length maintenance by generating the 3' overhang which serves as a primer for telomerase dependent telomere elongation. NBN is a major player in the control of intra-S-phase checkpoint and there is some evidence that NBN is involved in G1 and G2 checkpoints. The roles of NBS1/MRN encompass DNA damage sensor, signal transducer, and effector, which enable cells to maintain DNA integrity and genomic stability. Forms a complex with RBBP8 to link DNA double-strand break sensing to resection. Enhances AKT1 phosphorylation possibly by association with the mTORC2 complex.

Tissue specificity Ubiquitous. Expressed at high levels in testis.

Involvement in disease Nijmegen breakage syndrome

	Breast cancer Aplastic anemia Defects in NBN might play a role in the pathogenesis of childhood acute lymphoblastic leukemia (ALL).
Sequence similarities	Contains 1 BRCT domain. Contains 1 FHA domain.
Domain	The FHA and BRCT domains are likely to have a crucial role for both binding to histone H2AFX and for relocalization of MRE11/RAD50 complex to the vicinity of DNA damage. The C-terminal domain contains a MRE11-binding site, and this interaction is required for the nuclear localization of the MRN complex. The EEXXXDDL motif at the C-terminus is required for the interaction with ATM and its recruitment to sites of DNA damage and promote the phosphorylation of ATM substrates, leading to the events of DNA damage response.
Post-translational modifications	Phosphorylated by ATM in response of ionizing radiation, and such phosphorylation is responsible intra-S phase checkpoint control and telomere maintenance.
Cellular localization	Nucleus. Nucleus, PML body. Chromosome, telomere. Localizes to discrete nuclear foci after treatment with genotoxic agents.

Images



Western blot - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

All lanes : Anti-p95/NBS1 antibody [7E4C2] (ab181780) at 1/500 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : NBN knockout A549 cell lysate
Lane 3 : Wild-type A431 cell lysate
Lane 4 : NBN knockout A431 cell lysate

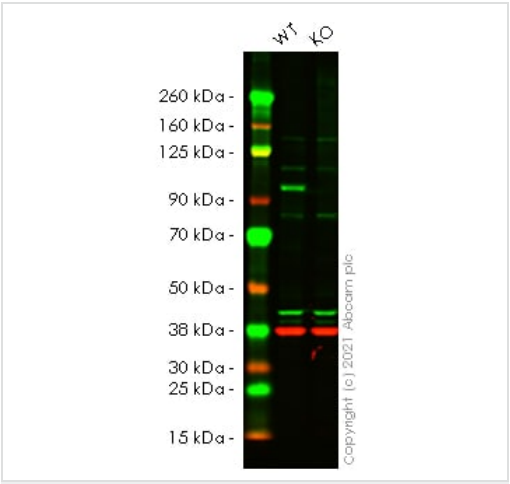
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 95 kDa

Anti-NBN antibody [7E4C2] (ab181780) staining at 1/500 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab181780 was shown to bind specifically to NBN. A band was observed at 95 kDa in wild-type A549 cell lysates with no signal observed at this size in NBN knockout cell line. To generate this image, wild-type and NBN knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then

transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

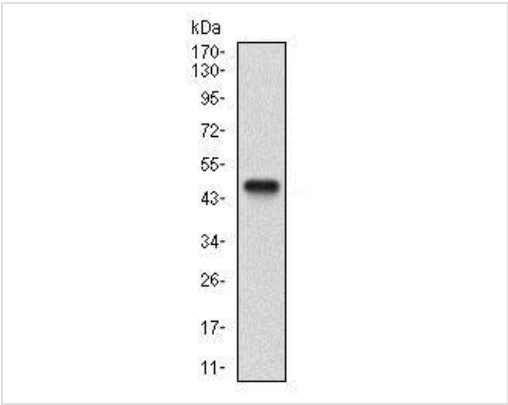
All lanes : Anti-p95/NBS1 antibody [7E4C2] (ab181780) at 1/500 dilution

Lane 1 : Wild-type A431 cell lysate
Lane 2 : NBN knockout A431 cell lysate

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 95 kDa

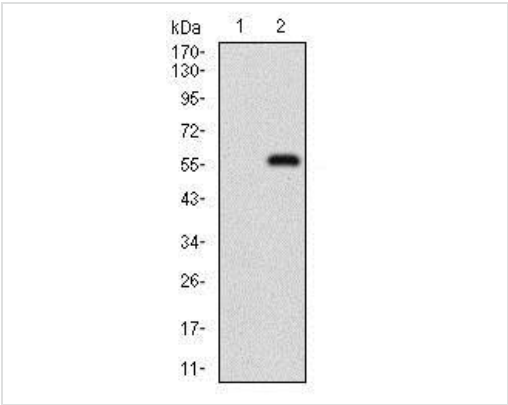
False colour image of Western blot: Anti-p95/NBS1 antibody [7E4C2] staining at 1/500 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab181780 was shown to bind specifically to p95/NBS1. A band was observed at 95 kDa in wild-type A431 cell lysates with no signal observed at this size in NBN knockout cell line **ab269506** (knockout cell lysate **ab269668**). To generate this image, wild-type and NBN knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDyeÂ® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

Anti-p95/NBS1 antibody [7E4C2] (ab181780) at 1/500 dilution + Human p95/NBS1 recombinant protein (amino acids 467-615)

Predicted band size: 85 kDa

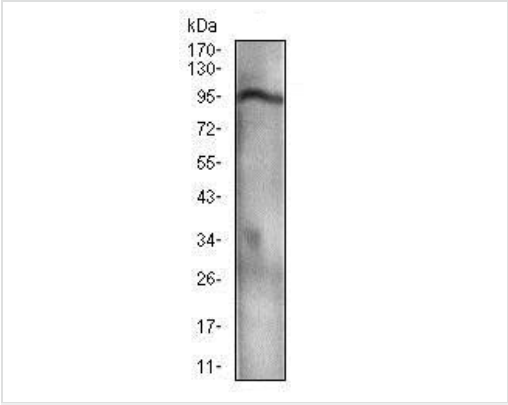


Western blot - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

All lanes : Anti-p95/NBS1 antibody [7E4C2] (ab181780) at 1/500 dilution

Lane 1 : HEK293 cell lysate, non-transfected
Lane 2 : HEK293 cell lysate, transfected with p95/NBS1 (amino acids 467-615)-hIgGFc

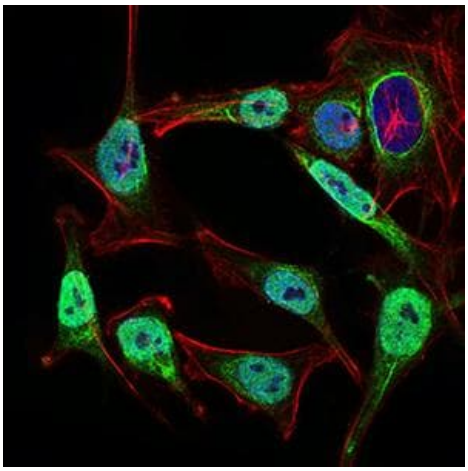
Predicted band size: 85 kDa



Western blot - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

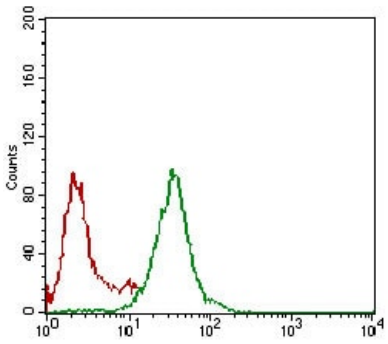
Anti-p95/NBS1 antibody [7E4C2] (ab181780) at 1/500 dilution + Jurkat cell lysate

Predicted band size: 85 kDa



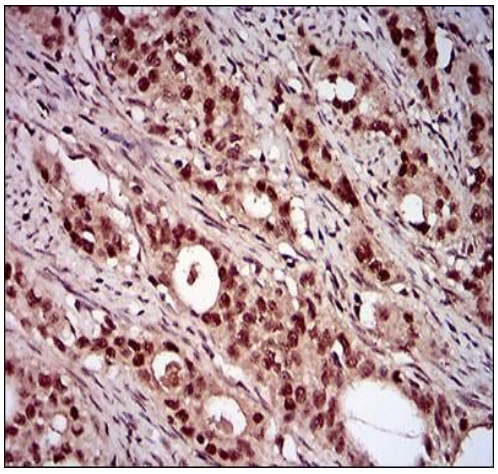
Immunocytochemistry/ Immunofluorescence - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

Immunofluorescent analysis of HeLa cells labeling p95/NBS1 with ab181780 at 1/200 dilution (green). Blue: DRAQ5 fluorescent DNA dye. Red: Actin filaments have been labeled with Alexa Fluor-555 phalloidin.



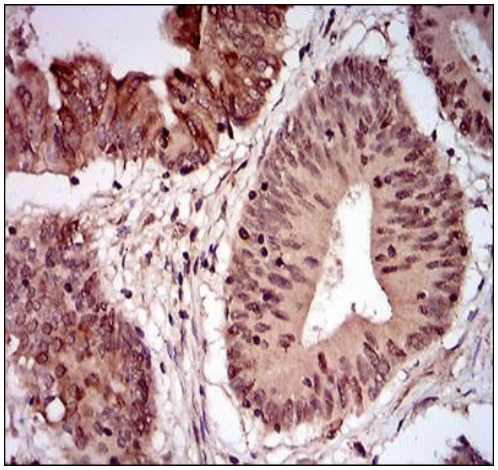
Flow Cytometry - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

Flow cytometric analysis of HeLa cells labeling p95/NBS1 with ab181780 at 1/200 dilution (green); negative control (red).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

Immunohistochemical analysis of paraffin-embedded Human cervical cancer tissue labeling p95/NBS1 with ab181780 at 1/200 dilution followed by DAB staining.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p95/NBS1 antibody [7E4C2] (ab181780)

Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labeling p95/NBS1 with ab181780 at 1/200 dilution followed by DAB staining.

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