abcam

Product datasheet

Anti-p95/NBS1 antibody ab23996

KO VALIDATED

★★★★★ 1 Abreviews 18 References 4 Images

Overview

Product name	Anti-p95/NBS1 antibody
Description	Rabbit polyclonal to p95/NBS1
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Chicken, Dog
Immunogen	Synthetic peptide corresponding to Human p95/NBS1 aa 700 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab24289**)
Positive control	WB: MRC5 whole cell lysate (SV40 transformed immortal (WT) fibroblasts), Hela whole cell, NIH3T3 whole cell, HepG2 whole cell and Jurkat whole cell, and A431 WT lysate ICC/IF: HeLa cells
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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: 99% PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab23996 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		1/1000 - 1/5000. Detects a band of approximately 95 kDa (predicted molecular weight: 85 kDa). Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.
ICC/IF	★★★★★ (1)	Use a concentration of 1 µg/ml.

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.

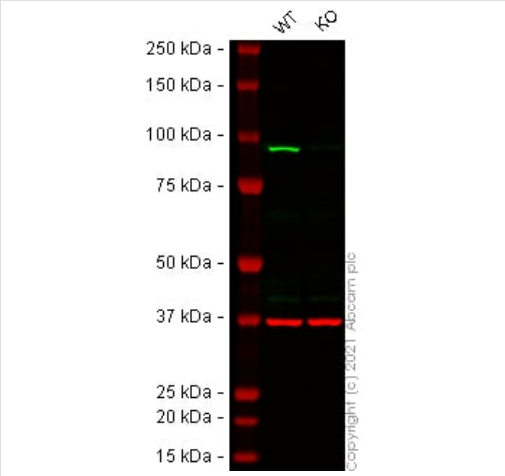
Tissue specificity Ubiquitous. Expressed at high levels in testis.

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 (ab23996)

All lanes : Anti-p95/NBS1 antibody (ab23996) at 1/1000 dilution

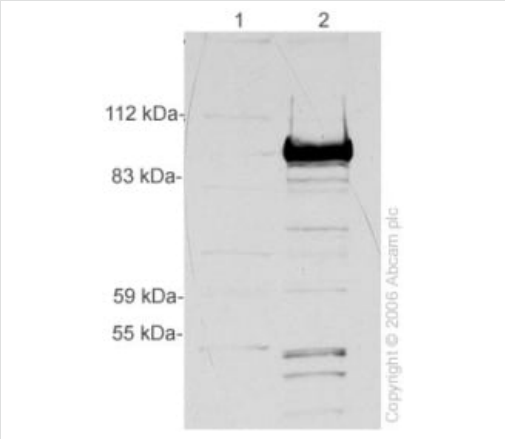
Lane 1 : Wild-type A431 cell lysate at 20 µg
Lane 2 : NBN knockout A431 cell lysate at 20 µg
Lane 3 : NBN knockout A431 cell lysate

Performed under reducing conditions.

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

False colour image of Western blot: Anti-p95/NBS1 antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab23996 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000

dilution.



Western blot - Anti-p95/NBS1 antibody (ab23996)

All lanes : Anti-p95/NBS1 antibody (ab23996) at 1/5000 dilution

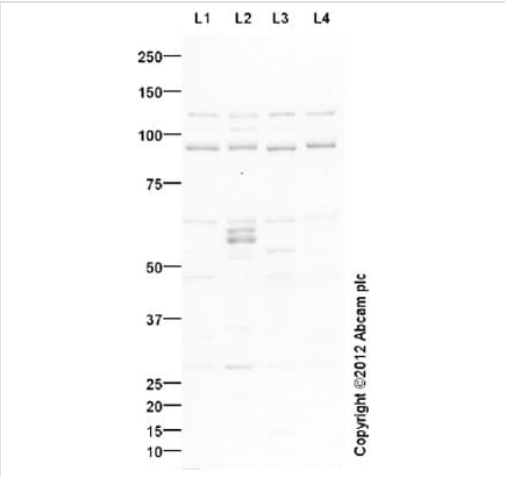
Lane 1 : Lysate from cells engineered to be NBS1 defective (SV40 transformed, immortal fibroblasts)
Lane 2 : MRC5 cell lysate (SV40 transformed immortal (WT) fibroblasts)

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

ab23996 detects a band at approximately 95 kDa, the size at which NBS1 migrates, in MRC5 cell lysate. This band is not detected in fibroblasts in which NBS1 is not expressed, indicating that it is specific for the NBS1 protein.



Western blot - Anti-p95/NBS1 antibody (ab23996)

All lanes : Anti-p95/NBS1 antibody (ab23996) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 4 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

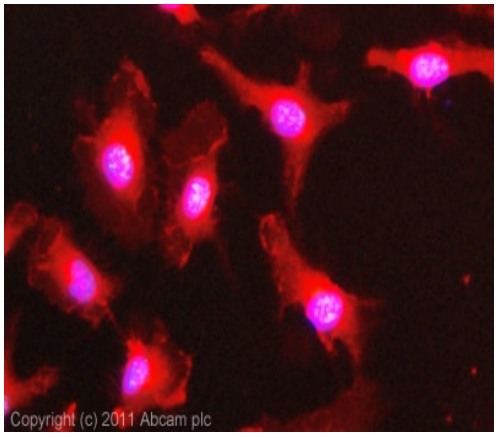
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 95 kDa
Additional bands at: 125 kDa, 55 kDa, 65 kDa. We are unsure as to the identity of these extra bands.



Immunocytochemistry/ Immunofluorescence - Anti-p95/NBS1 antibody (ab23996)

ICC/IF image of ab23996 stained HeLa 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 (ab23996, 1µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit 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. This antibody also gave a positive result in 100% methanol fixed (5 min) HepG2 and MCF7 cells at 1µg/ml.

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