abcam

Product datasheet

Human NBN (p95/NBS1) knockout A-431 cell lysate ab269668

5 Images

Overview

Product name	Human NBN (p95/NBS1) knockout A-431 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp deletion, 11 bp deletion; Frameshift: 99%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab280543 - Human NBN knockout A431 cell lysate	1 x 100µg
ab263973 - Human wild-type A-431 cell lysate	1 x 100µg

Cell type epithelial

Disease Epidermoid Carcinoma

Gender Female

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.

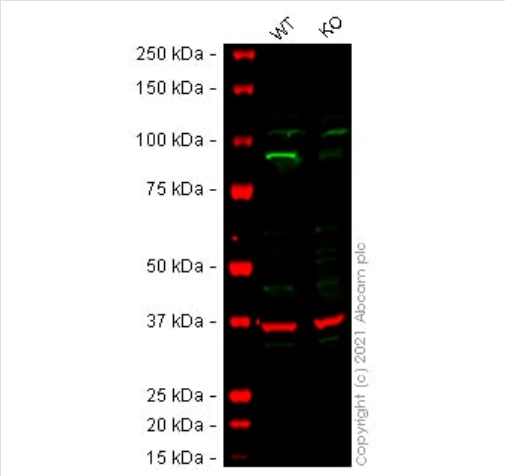
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab269668 in the following tested applications.

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

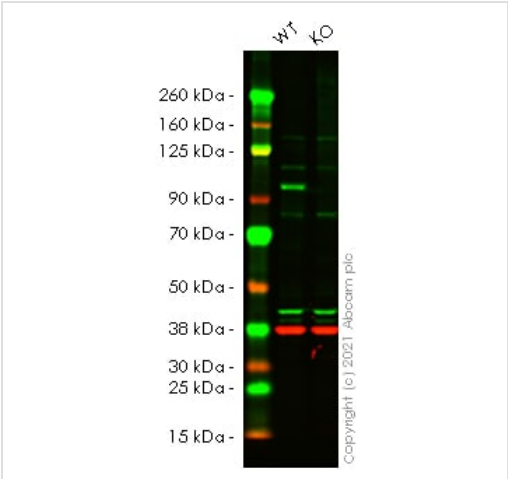
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

Images



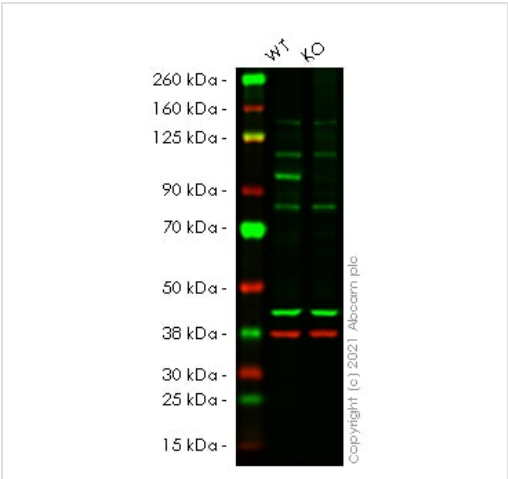
Western blot - Human NBN (p95/NBS1) knockout A-431 cell lysate (ab269668)

Lane 1: Wild-type A431 cell lysate 20 µg
Lane 2: NBN knockout A431 cell lysate 0 µg
Lane 3: NBN knockout A431 cell lysate 20 µg
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 - Human NBN (p95/NBS1) knockout A-431 cell lysate (ab269668)

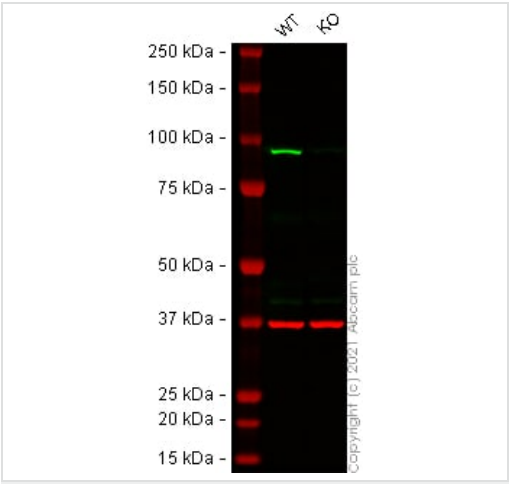
Lane 1: Wild-type A431 cell lysate 20 µg
Lane 2: NBN knockout A431 cell lysate 20 µg
False colour image of Western blot: Anti-p95/NBS1 antibody [7E4C2] staining at 1/500 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown 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 (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Human NBN (p95/NBS1) knockout A-431 cell lysate (ab269668)

Lane 1: Wild-type A431 cell lysate 20 µg
Lane 2: NBN knockout A431 cell lysate 20 µg
False colour image of Western blot: Anti-p95/NBS1 antibody [7E4A2] 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, **ab181729** 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 (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Human NBN (p95/NBS1) knockout A-431 cell lysate (ab269668)

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

Lanes 1 - 2: Merged signal (red and green). Green - **ab32074** observed at 90 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab32074 was shown to react with p95/NBS1 in wild-type A431 cells in Western blot with loss of signal observed in NBN knockout cell line **ab269506** (NBN knockout cell lysate ab269668). Wild-type A431 and NBN knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5 % milk in TBS-T (0.1 % Tween®) before incubation with **ab32074** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Next Generation Sequencing - Human NBN (p95/NBS1) knockout A-431 cell lysate (ab269668)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp deletion, 11 bp deletion; Frameshift: 99%

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