abcam

Product datasheet

Human NBN (p95/NBS1) knockout HeLa cell lysate ab257111

3 Images

Overview

Product name	Human NBN (p95/NBS1) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 16 bp deletion in exon2 and 1 bp insertion in exon2.
Passage number	<20
Knockout validation	Sanger Sequencing, 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:** Sanger Sequencing, WB

Properties

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

Components	1 kit
ab260919 - Human NBN knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

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.

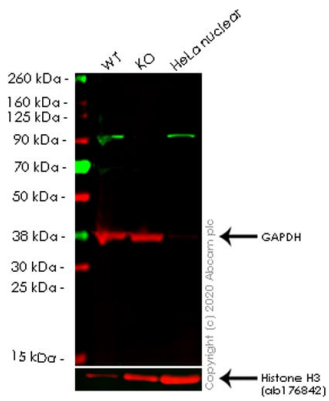
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257111 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
Sanger Sequencing		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.

Images



Western blot - Human NBN knockout HeLa cell lysate (ab257111)

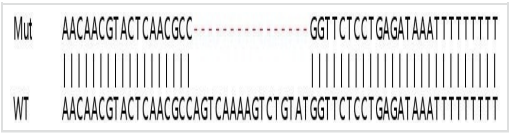
Lane 1: Wild-type HeLa cell lysate (20 µg)

Lane 2: NBN knockout HeLa cell lysate (20 µg)

Lane 3: Wild-type HeLa nuclear cell lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - **ab32074** observed at 95 kDa.

ab32074 Anti-p95/NBS1 antibody [Y112] was shown to specifically react with p95/NBS1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261834** (knockout cell lysate ab257111) was used. Wild-type and p95/NBS1 knockout samples were subjected to SDS-PAGE. **ab32074**, Anti-GAPDH antibody [6C5] - Cytoplasmic Loading Control (**ab8245**) and Anti-Histone H3 (**ab176842**) - Nuclear Loading Control were incubated overnight at 4°C at 1 in 1000 dilution, 1 in 20000 dilution and 1 in 1000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**), Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human NBN knockout HeLa cell lysate (ab257111)

Allele-1: 16 bp deletion in exon2

Sanger Sequencing - Human NBN knockout HeLa cell lysate (ab257111)

Allele-2: 1 bp insertion in exon2

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