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Anti-p95 NBS1 Picoband Antibody

Catalog # ABO12301

Product Information

Application	WB, IHC-P, ICC
Primary Accession	O60934
Host	Rabbit
Reactivity	Human, Mouse, Rat
Clonality	Polyclonal
Format	Lyophilized
Description	Rabbit IgG polyclonal antibody for Nibrin(NBN) detection. Tested with WB, IHC-P, ICC in Human;Mouse;Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	4683
Other Names	Nibrin, Cell cycle regulatory protein p95, Nijmegen breakage syndrome protein 1, NBN, NBS, NBS1, P95
Calculated MW	84959
Application Details	Immunohistochemistry(Paraffin-embedded Section), 0.5-1 □g/ml, By Heat Immunocytochemistry, 0.5-1 □g/ml Western blot, 0.1-0.5 □g/ml
Subcellular Localization	Nucleus . Nucleus, PML body . Chromosome, telomere . Localizes to discrete nuclear foci after treatment with genotoxic agents. .
Tissue Specificity	Ubiquitous. Expressed at high levels in testis.
Source	Eukaryota
Protein Name	Nibrin
Contents	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human p95 NBS1 (714-745aa RKNTELEEWLRQEMEVQNQHAKEESLADDLFR), different from the related mouse sequence by three amino acids, and from the related rat sequence by five amino acids.
Purification	Immunogen affinity purified.

Cross Reactivity	No cross reactivity with other proteins
Storage	At -20˚C for one year. After r˚Constitution, at 4˚C for one month. It˚Can also be aliquotted and stored frozen at -20˚C for a longer time.Avoid repeated freezing and thawing.
Sequence Similarities	Contains 1 BRCT domain.

Protein Information

Name	NBN (HGNC:7652)
Function	Component of the MRN complex, which plays a central role in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis (PubMed:10888888, PubMed:15616588, PubMed:18411307, PubMed:18583988, PubMed:18678890, PubMed:19759395, PubMed:23115235, PubMed:28216226, PubMed:28867292, PubMed:9705271). The MRN complex is involved in the repair of DNA double-strand breaks (DSBs) via homologous recombination (HR), an error-free mechanism which primarily occurs during S and G2 phases (PubMed:19759395, PubMed:28867292, PubMed:9705271). The complex (1) mediates the end resection of damaged DNA, which generates proper single-stranded DNA, a key initial steps in HR, and is (2) required for the recruitment of other repair factors and efficient activation of ATM and ATR upon DNA damage (PubMed:19759395, PubMed:9705271). The MRN complex possesses single-strand endonuclease activity and double-strand-specific 3'-5' exonuclease activity, which are provided by MRE11, to initiate end resection, which is required for single-strand invasion and recombination (PubMed:19759395, PubMed:28867292, PubMed:9705271). Within the MRN complex, NBN acts as a protein-protein adapter, which specifically recognizes and binds phosphorylated proteins, promoting their recruitment to DNA damage sites (PubMed:12419185, PubMed:15616588, PubMed:18411307, PubMed:18582474, PubMed:18583988, PubMed:18678890, PubMed:19759395, PubMed:19804756, PubMed:23762398, PubMed:24534091, PubMed:27814491, PubMed:27889449, PubMed:33836577). Recruits MRE11 and RAD50 components of the MRN complex to DSBs in response to DNA damage (PubMed:12419185, PubMed:18411307, PubMed:18583988, PubMed:18678890, PubMed:24534091, PubMed:26438602). Promotes the recruitment of PI3/PI4-kinase family members ATM, ATR, and probably DNA-PKcs to the DNA damage sites, activating their functions (PubMed:15064416, PubMed:15616588, PubMed:15790808, PubMed:16622404, PubMed:22464731, PubMed:30952868, PubMed:35076389). Mediates the recruitment of phosphorylated RBBP8/CtIP to DSBs, leading to cooperation between the MRN complex and RBBP8/CtIP to initiate end resection (PubMed:19759395, PubMed:27814491, PubMed:27889449, PubMed:33836577). RBBP8/CtIP specifically promotes the endonuclease activity of the MRN complex to clear DNA ends containing protein adducts (PubMed:27814491, PubMed:27889449, PubMed:30787182, PubMed:33836577). The MRN complex is also required for the processing of R-loops (PubMed:31537797). NBN also functions in telomere length maintenance via its interaction with TERF2: interaction with TERF2 during G1 phase preventing recruitment of DCLRE1B/Apollo to telomeres (PubMed:10888888, PubMed:28216226). NBN also promotes DNA repair choice at dysfunctional telomeres: NBN phosphorylation by CDK2 promotes non- homologous end joining repair at telomeres, while unphosphorylated NBN promotes microhomology-mediated end-joining (MMEJ) repair (PubMed:28216226). Enhances AKT1 phosphorylation possibly by association with the mTORC2 complex (PubMed:23762398).

Cellular Location	Nucleus. Chromosome. Nucleus, PML body. Chromosome, telomere Note=Localizes to discrete nuclear foci after treatment with genotoxic agents (PubMed:10783165, PubMed:26215093, PubMed:26438602). Localizes to DNA double-strand breaks (DSBs); recruited to DNA damage sites via association with phosphorylated proteins, such as phosphorylated H2AX, phosphorylated MDC1 and phosphorylated RAD17 (PubMed:12419185, PubMed:18411307, PubMed:18582474, PubMed:18583988, PubMed:18678890, PubMed:19338747, PubMed:23115235, PubMed:24534091, PubMed:26438602) Acetylation of 'Lys-5' of histone H2AX (H2AXK5ac) promotes NBN/NBS1 assembly at the sites of DNA damage (PubMed:26438602)
Tissue Location	Ubiquitous (PubMed:9590180). Expressed at high levels in testis (PubMed:9590180).

Background

p95 NBS1, also known as NBN or Nibrin, is a protein which in humans is encoded by the NBN gene. Nibrin is a protein associated with the repair of double strand breaks (DSBs) which pose serious damage to a genome. It is a 754 amino acid protein identified as a member of the NBS1/hMre11/RAD50(N/M/R, more commonly referred to asMRN) double strand DNA break repair complex. This complex recognizes DNA damage and rapidly relocates to DSB sites and forms nuclear foci. It also has a role in regulation of N/M/R (MRN) protein complex activity which includes end-processing of both physiological and mutagenic DNA double strand breaks (DSBs).

Images

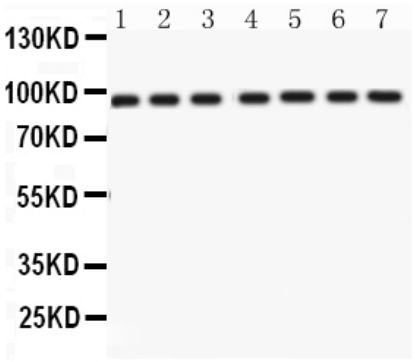
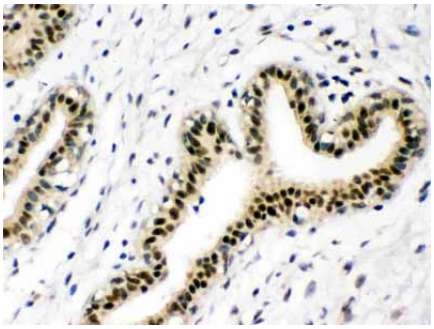


Figure 1. Western blot analysis of p95 NBS1 using anti-p95 NBS1 antibody (ABO12301). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: Rat Testis Tissue Lysate,Lane 2: Rat Brain Tissue Lysate,Lane 3: Rat Liver Tissue Lysate,Lane 4: Mouse Testis Tissue Lysate,Lane 5: HELA Whole Cell Lysate,Lane 6: A431 Whole Cell Lysate,Lane 7: HUT Whole Cell Lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-p95 NBS1 antigen affinity purified polyclonal antibody (Catalog # ABO12301) at 0.5 □¼g/mL overnight at 4 □°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for p95 NBS1 at approximately 95KD. The expected band size for p95 NBS1 is at 95KD.

Figure 2. IHC analysis of p95 NBS1 using anti-p95 NBS1 antibody (ABO12301).p95 NBS1 was detected in paraffin-embedded section of Human Mammary Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat



serum. The tissue section was then incubated with 1 □¼g/ml rabbit anti-p95 NBS1 Antibody (ABO12301) overnight at 4 □°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 □°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

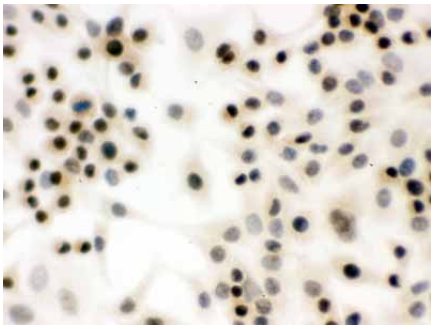


Figure 3. IHC analysis of p95 NBS1 using anti-p95 NBS1 antibody (ABO12301).p95 NBS1 was detected in immunocytochemical section of A549 cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 □¼g/ml rabbit anti-p95 NBS1 Antibody (ABO12301) overnight at 4 □°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 □°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



Figure 4. IHC analysis of p95 NBS1 using anti-p95 NBS1 antibody (ABO12301).p95 NBS1 was detected in immunocytochemical section of SMMC-7721 cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 □¼g/ml rabbit anti-p95 NBS1 Antibody (ABO12301) overnight at 4 □°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 □°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

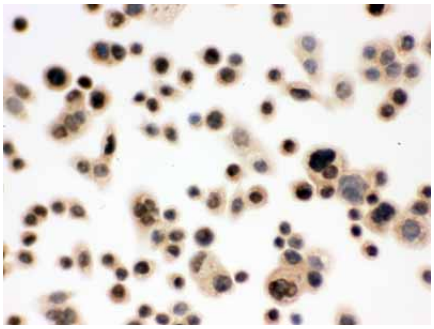


Figure 5. IHC analysis of p95 NBS1 using anti-p95 NBS1 antibody (ABO12301).p95 NBS1 was detected in immunocytochemical section of SW480 cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 □¼g/ml rabbit anti-p95 NBS1 Antibody (ABO12301) overnight at 4 □°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 □°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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