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Anti-p95 NBS1 Antibody Picoband™ (monoclonal, 5D7A1)

Catalog # ABO15096

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	O60934
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-p95 NBS1 Antibody Picoband™ (monoclonal, 5D7A1) . Tested in FCM, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	4683
Other Names	Nibrin, Cell cycle regulatory protein p95, Nijmegen breakage syndrome protein 1, hNbs1, NBN (HGNC:7652)
Calculated MW	84959
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 5D7A1
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human p95 NBS1, different from the related mouse sequence by three amino acids, and from the related rat sequence by five amino acids.
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

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

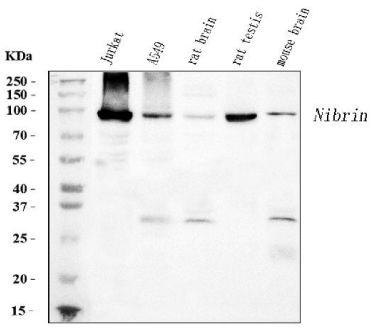


Figure 1. Western blot analysis of p95 NBS1 using anti-p95 NBS1 antibody (M00732-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Jurkat whole cell lysates,
Lane 2: human A549 whole cell lysates,
Lane 3: rat brain tissue lysates,
Lane 4: rat testis tissue lysates,
Lane 5: mouse brain tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-p95 NBS1 antigen affinity purified monoclonal antibody (Catalog # M00732-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for p95 NBS1 at approximately 95 kDa. The expected band size for p95 NBS1 is at 95 kDa.

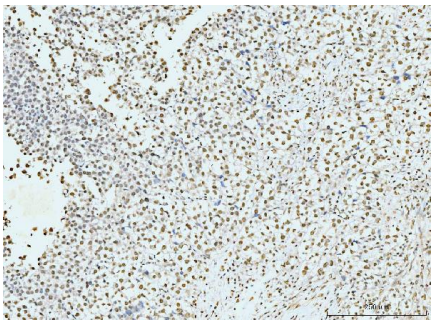


Figure 2. IHC analysis of p95 NBS1 using anti-p95 NBS1 antibody (M00732-1).
p95 NBS1 was detected in a paraffin-embedded section of human testis cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-p95 NBS1 Antibody (M00732-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30

minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

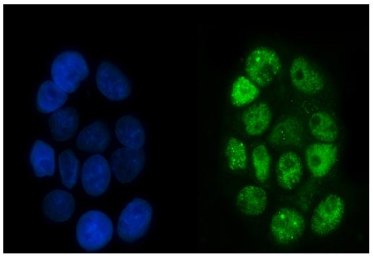


Figure 3. IF analysis of p95 NBS1 using anti-p95 NBS1 antibody (M00732-1).
p95 NBS1 was detected in an immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-p95 NBS1 Antibody (M00732-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

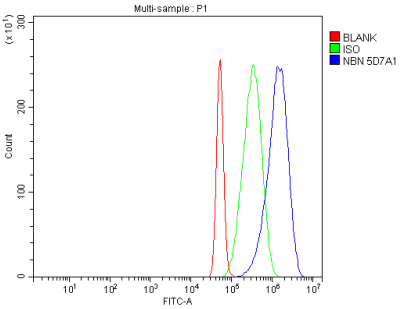


Figure 4. Flow Cytometry analysis of A431 cells using anti-p95 NBS1 antibody (M00732-1).
Overlay histogram showing A431 cells stained with M00732-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-p95 NBS1 Antibody (M00732-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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