# abcam

#### Product datasheet

## Anti-p95/NBS1 antibody [Y112] ab32074



★★★★ 5 Abreviews 25 References 画像数7

#### 製品の概要

製品名 Anti-p95/NBS1 antibody [Y112]

製品の詳細 Rabbit monoclonal [Y112] to p95/NBS1

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P

適用なし: Flow Cyt or ICC/IF

種交差性 交差種: Human

交差が予測される動物種: Mouse, Rat 4

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HeLa, Jurkat and Wild-type A431 cell lysates. IHC-P: Human testis and skin carcinoma

tissues.

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### 製品の特性

製品の状態

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

**クローン名** Y112

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32074の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	<b>★★★★</b> ★ (3)	1/1000 - 1/10000. Predicted molecular weight: 85 kDa. For unpurified use at 1/1000 - 1/2000.
IHC-P	****(1)	1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/50.

追加情報

Is unsuitable for Flow Cyt or ICC/IF.

#### ターゲット情報

機能

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 Pl3/Pl4-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.

組織特異性

Ubiquitous. Expressed at high levels in testis.

関連疾患

Nijmegen breakage syndrome

Breast cancer Aplastic anemia

Defects in NBN might play a role in the pathogenesis of childhood acute lymphoblastic leukemia

(ALL).

配列類似性

Contains 1 BRCT domain.

Contains 1 FHA domain.

ドメイン

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.

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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.

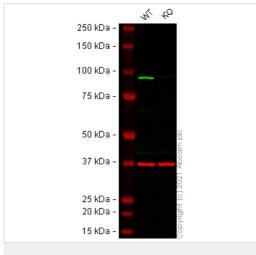
Phosphorylated by ATM in response of ionizing radiation, and such phosphorylation is responsible intra-S phase checkpoint control and telomere maintenance.

Nucleus. Nucleus, PML body. Chromosome, telomere. Localizes to discrete nuclear foci after treatment with genotoxic agents.

#### 画像

翻訳後修飾

細胞内局在



Western blot - Anti-p95/NBS1 antibody [Y112] (ab32074)

**All lanes :** Anti-p95/NBS1 antibody [Y112] (ab32074) at 1/1000 dilution

Lane 1 : Wild-type A431 cell lysate

Lane 2 : NBN knockout A431 cell lysate

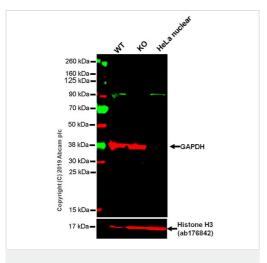
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa Observed band size: 90 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab32074 observed at 90 kDa. Red - loading control <u>ab8245</u> (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 <a href="mailto:ab269506">ab269506</a> (NBN knockout cell lysate <a href="mailto:ab269668">ab269668</a>). 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 <a href="mailto:ab8245">ab8245</a> (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 (<a href="mailto:ab216776">ab216773</a>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-p95/NBS1 antibody [Y112] (ab32074)

**All lanes :** Anti-p95/NBS1 antibody [Y112] (ab32074) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: NBN knockout HeLa cell lysate

Lane 3: Wild-type HeLa nuclear cell lysate

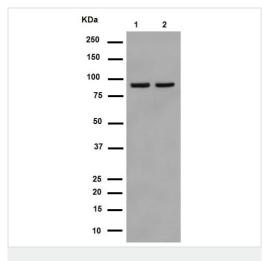
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**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 <a href="mailto:ab261834">ab261834</a> (knockout cell lysate <a href="mailto:ab257111">ab261834</a> (knockout cell lysate <a href="mailto:ab257111">ab267111</a>) 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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773), Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-p95/NBS1 antibody [Y112] (ab32074)

**All lanes :** Anti-p95/NBS1 antibody [Y112] (ab32074) at 1/1200 dilution (unpurified)

Lane 1 : HeLa cell lysate

Lane 2 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

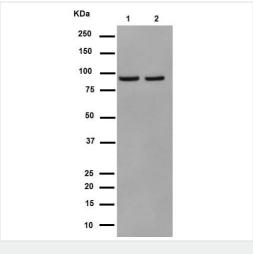
Predicted band size: 85 kDa Observed band size: 95 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p95/NBS1 antibody
[Y112] (ab32074)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue labelling p95/NBS1 with purified ab32074 at 1/200. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Counterstained with hematoxylin.



Western blot - Anti-p95/NBS1 antibody [Y112] (ab32074)

**All lanes :** Anti-p95/NBS1 antibody [Y112] (ab32074) at 1/10000 dilution (purified)

Lane 1 : HeLa cell lysate

Lane 2 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

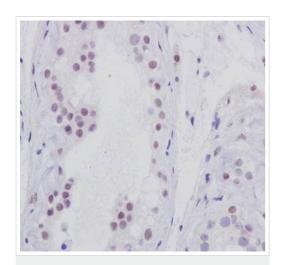
### Secondary

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

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

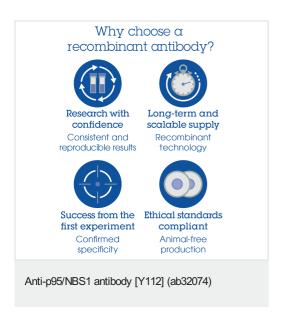
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p95/NBS1 antibody
[Y112] (ab32074)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue labelling p95/NBS1 with unpurified ab32074 at 1/20. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Counterstained with hematoxylin.



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