abcam

Product datasheet

Anti-Bax antibody [E63] ab32503

KO 評価済 リコンビナント RabMAb

12 Abreviews 2098 References 画像数 12

製品の概要

製品名	Anti-Bax antibody [E63]
製品の詳細	Rabbit monoclonal [E63] to Bax
由来種	Rabbit
特異性	Expression levels of BAX protein vary with sample type. Induction may be required if endogenous expression is low.
アプリケーション	**適用あり:** IHC-P, WB, IP, Sandwich ELISA **適用なし:** Flow Cyt or ICC/IF
種交差性	**交差種:** Mouse, Rat, Human **交差が予測される動物種:** Cow
免疫原	Synthetic peptide within Human Bax aa 1-100 (N terminal). The exact sequence is proprietary. Database link: **Q07812** (Peptide available as **ab188834**)
ポジティブ・コントロール	WB: Recombinant Human Bax protein (Tagged) (**ab85157**), HeLa, HepG2, A549, C2C12 and C6 cell lysate. Wild-type Hap1 cell lysate. Rat spleen tissue lysate. IHC-P: Human lymph node and rat kidney tissues. Human lung carcinoma tissue. IP: HeLa cell lysate.
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	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 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	E63
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab32503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
IHC-P	★★★★☆ (2)	1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★★ (8)	1/1000 - 1/10000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IP		1/100.
Sandwich ELISA		Use at an assay dependent concentration.

追加情報 Is unsuitable for Flow Cyt or ICC/IF.

ターゲット情報

機能 Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19k protein. Under stress conditions, undergoes a conformation change that causes translocation to the mitochondrion membrane, leading to the release of cytochrome c that then triggers apoptosis. Promotes activation of CASP3, and thereby apoptosis.

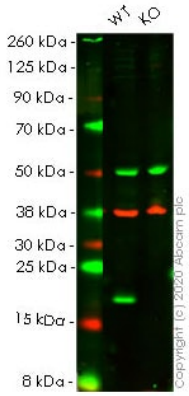
組織特異性 Expressed in a wide variety of tissues. Isoform Psi is found in glial tumors. Isoform Alpha is expressed in spleen, breast, ovary, testis, colon and brain, and at low levels in skin and lung. Isoform Sigma is expressed in spleen, breast, ovary, testis, lung, colon, brain and at low levels in skin. Isoform Alpha and isoform Sigma are expressed in pro-myelocytic leukemia, histiocytic lymphoma, Burkitt's lymphoma, T-cell lymphoma, lymphoblastic leukemia, breast adenocarcinoma, ovary adenocarcinoma, prostate carcinoma, prostate adenocarcinoma, lung carcinoma, epidermoid carcinoma, small cell lung carcinoma and colon adenocarcinoma cell lines.

配列類似性 Belongs to the Bcl-2 family.

ドメイン Intact BH3 motif is required by BIK, BID, BAK, BAD and BAX for their pro-apoptotic activity and for their interaction with anti-apoptotic members of the Bcl-2 family.

細胞内局在 Cytoplasm and Mitochondrion membrane. Cytoplasm. Colocalizes with 14-3-3 proteins in the cytoplasm. Under stress conditions, undergoes a conformation change that causes release from JNK-phosphorylated 14-3-3 proteins and translocation to the mitochondrion membrane.

画像



Western blot - Anti-Bax antibody [E63] (ab32503)

All lanes : Anti-Bax antibody [E63] (ab32503) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BAX knockout HeLa cell lysate

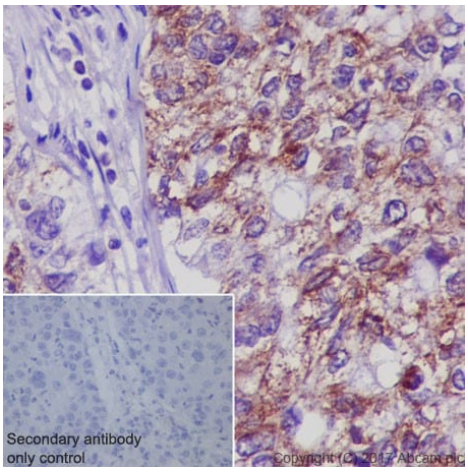
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 21 kDa

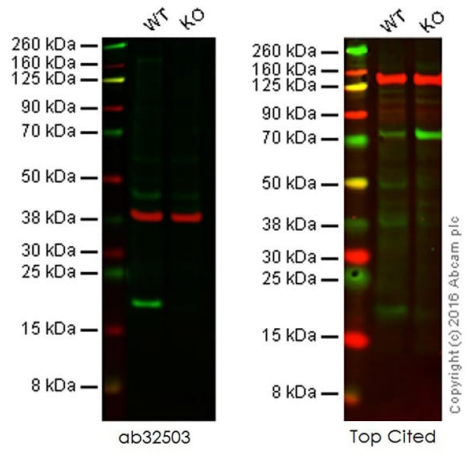
Lanes 1- 2: Merged signal (red and green). Green - ab32503 observed at 21 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32503 was shown to react with Bax in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255363** (knockout cell lysate **ab263841**) was used. Wild-type HeLa and BAX knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32503 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bax antibody [E63] (ab32503)

Purified ab32503 staining Bax in Human lung carcinoma tissue section by immmunohistochemistry (IHC-P- Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with paraffin and heat mediated antigen retrieval was performed using EDTA buffer (pH 9.0). Samples were incubated with primary antibody at 1:500 dilution. A goat anti-rabbit IgG H&L (HRP) (**ab97051**) was used as a secondary antibody at 1:500 dilution. Cytoplasmic staining on human lung carcinoma.



Western blot - Anti-Bax antibody [E63] (ab32503)

All lanes : Anti-Bax antibody [E63] (ab32503)

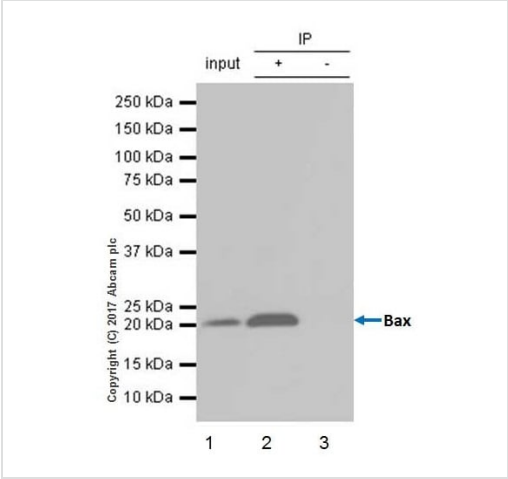
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Bax knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 21 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab32503 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37 kDa or **ab18058**, observed at 130 kDa.

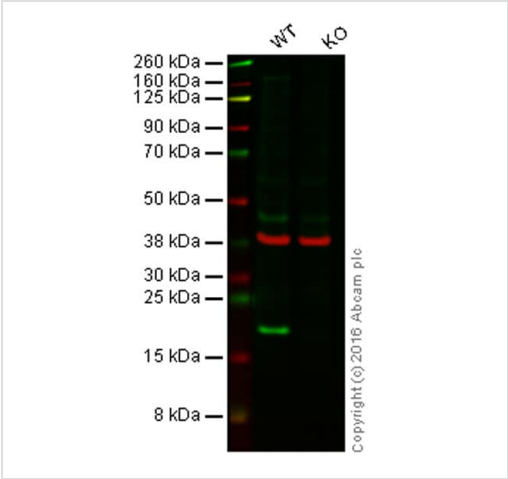
This western blot image is a comparison between ab32503 and a competitor's top cited rabbit polyclonal antibody.



Immunoprecipitation - Anti-Bax antibody [E63] (ab32503)

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, 10µg
Lane 2 (+): HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32503 in HeLa whole cell lysate

Purified ab32503 immunoprecipitating Bax in HeLa lysates. For western blotting, the primary antibody used was purified ab32503 at 1/1000 dilution. Ab131366 VeriBlot for IP Detection Reagent (HRP) was used for detection at 1/1000 dilution. Capture antibody was used at a 1/20 dilution. Blocking and diluting buffer used was 5% NFDM/TBST.



Western blot - Anti-Bax antibody [E63] (ab32503)

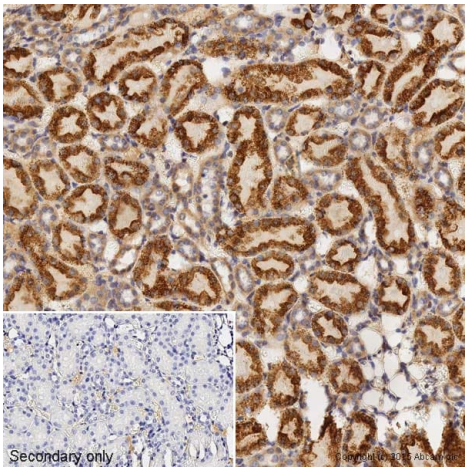
All lanes : Anti-Bax antibody [E63] (ab32503) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Bax knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

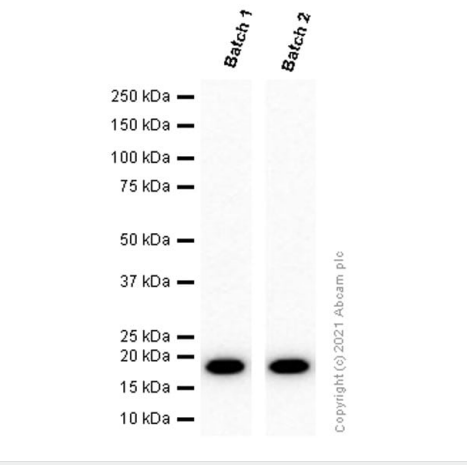
Predicted band size: 21 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab32503 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab32503 was shown to recognize Bax in wild-type HAP1 cells, along with additional cross-reactive bands. Wild-type and Bax knockout samples were subjected to SDS-PAGE. ab32503 and **ab8245** (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



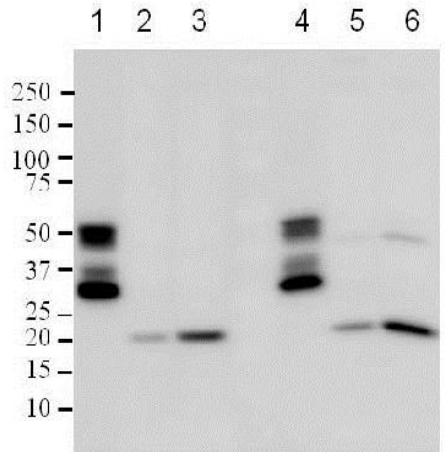
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bax antibody [E63] (ab32503)

IHC image of ab32503 staining Bax in rat kidney formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32503, 1:250 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



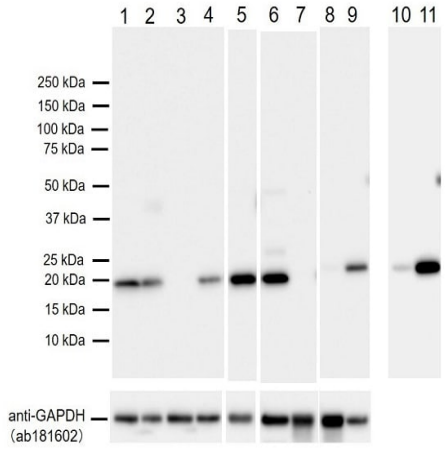
Western blot - Anti-Bax antibody [E63] (ab32503)

Different batches of ab32503 were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 0.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 18 kDa.



Western blot - Anti-Bax antibody [E63] (ab32503)

Lane 1 = Bax protein (Tagged) (**ab85157**), 10 ng. Lane 2 = Extract of HeLa cells, 40 ug. Lane 3 = Extract of HepG2 cells, 40 ug. Lane 4 = Bax protein (Tagged) (**ab85157**), 10 ng. Lane 5 = Extract of HeLa cells, 40 ug. Lane 6 = Extract of HepG2 cells, 40 ug. SDS PAGE performed under reducing conditions (100mM DTT Sample heated at 50°C). Primary : Lanes 1-3: Anti Bax antibody (**ab77566**) at 1 ug/mL. Lanes 4-6: Anti Bax antibody (ab32503) at 1:2000 dilution. Secondary : Lanes 1-3: Goat anti mouse IgG(H&L)-HRP at 1:10000. Lanes 4-6: Goat anti rabbit IgG(H&L)-HRP at 1:10000. Development: ECL with 2 min exposure. Blocking: in 5% Milk + PBS for 3 hours at RT. Primary antibody: in 5% Milk + PBS overnight at 4 C. Secondary antibody: in 5% Milk + PBS for 2 hour at RT. Predicted band size : Bax 21kDa and Bax (Tagged) 49 kDa. Observed band size : Bax 21kDa and Bax (Tagged) 49 kDa.



Western blot - Anti-Bax antibody [E63] (ab32503)

All lanes : Anti-Bax antibody [E63] (ab32503) at 1/2000 dilution (purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell). Whole cell lysates
Lane 2 : Hep G2 (Human hepatocellular carcinoma epithelial cell). Whole cell lysates
Lane 3 : Jurkat (Human T cell leukemia T lymphocyte) Whole cell lysates
Lane 4 : A549 (Human lung carcinoma epithelial cell) Whole cell lysates
Lane 5 : C2C12 (Mouse myoblasts myoblast) Whole cell lysates
Lane 6 : C6 (Rat glial tumor glial cell) Whole cell lysates
Lane 7 : Mouse brain. Whole tissue lysate
Lanes 8 & 10 : Rat brain. Whole tissue lysate
Lanes 9 & 11 : Rat Spleen. Whole tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

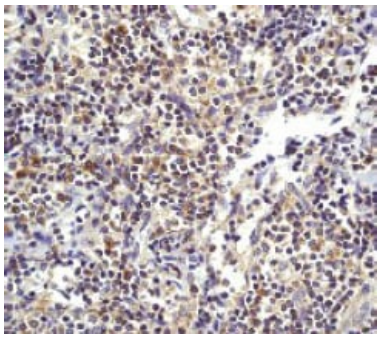
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 21 kDa
Observed band size: 18 kDa

Blocking and Diluting buffers: 5% NFDM/TBST

Exposure time 1~9 lanes 32 s; 10~11 lanes 3 min

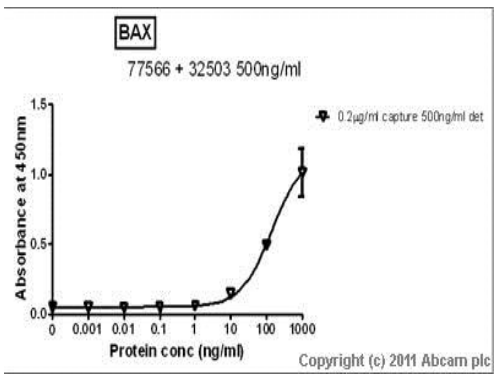
Jurkat is negative reported by PMID: 15528359. Brain is low expressed reported by PMID: 27069530.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bax antibody [E63] (ab32503)

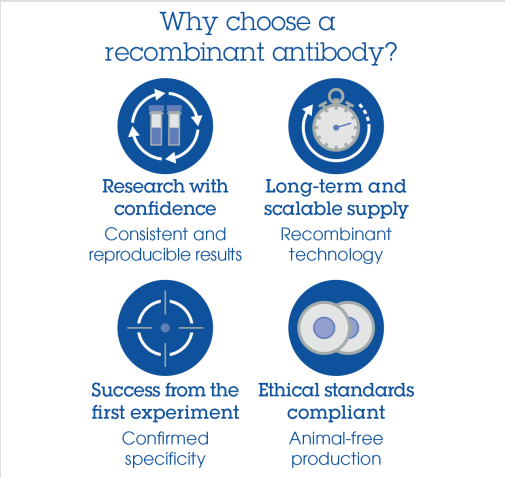
Immunohistochemical analysis of paraffin-embedded human lymph node using anti-Bax Rabbit Monoclonal Antibody (ab32503) at 1/250 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Sandwich ELISA - Anti-Bax antibody [E63] (ab32503)

Standard Curve for Bax (Analyte: Recombinant human Bax protein (tagged) **ab85157**) dilution range 1pg/ml to 1ug/ml using Capture Antibody **Mouse monoclonal [2D2] to Bax - BSA and Azide free (ab77566)** at 0.2ug/ml and Detector Antibody **Rabbit monoclonal [E63] to Bax (ab32503)** at 0.5ug/ml Concentration of **ab32503** may vary from lot to lot; please use this curve as guideline.



Anti-Bax antibody [E63] (ab32503)

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