

## Product datasheet

# Anti-Bax antibody [E63] ab32503

**KO VALIDATED** Recombinant RabMAB<sup>®</sup>

★★★★★ 15 Abreviews 255 References 9 Images

### Overview

<b>Product name</b>	Anti-Bax antibody [E63]
<b>Description</b>	Rabbit monoclonal [E63] to Bax
<b>Host species</b>	Rabbit
<b>Specificity</b>	Expression levels of BAX protein vary with sample type. Induction may be required if endogenous expression is low.
<b>Tested applications</b>	<b>Suitable for:</b> IHC-P, WB, IP, Sandwich ELISA <b>Unsuitable for:</b> Flow Cyt or ICC
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human, Chinese hamster <b>Predicted to work with:</b> Cow
<b>Immunogen</b>	Synthetic peptide within Human Bax aa 1-100 (N terminal). The exact sequence is proprietary. <a href="#">Run BLAST with ExPASy</a> <a href="#">Run BLAST with NCBI</a>
<b>Positive control</b>	WB: Recombinant Human Bax protein (Tagged) ( <a href="#">ab85157</a> ), HeLa cell lysate. IHC-P: Human lymph node and rat kidney tissues.
<b>General notes</b>	Our RabMAB <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMab<sup>®</sup> patents</a> .  This product is a <a href="#">recombinant rabbit monoclonal antibody</a> .  A trial size is available to purchase for this antibody.

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	E63

**Isotype** IgG

## Applications

Our [Abpromise guarantee](#) covers the use of **ab32503** 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
IHC-P	★★★★★	1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★★	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. Can be paired for Sandwich ELISA with <a href="#">Mouse monoclonal [2D2] to Bax (ab77566)</a> .

**Application notes** Is unsuitable for Flow Cyt or ICC.

## Target

**Function** 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.

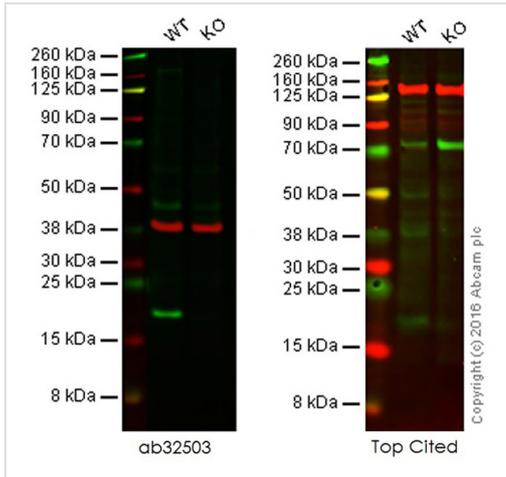
**Tissue specificity** Expressed in a wide variety of tissues. Isoform Psi is found in glial tumors. Isoform Alpha is expressed in spleen, breast, ovary, testis, colon and brain, and at low levels in skin and lung. Isoform Sigma is expressed in spleen, breast, ovary, testis, lung, colon, brain and at 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.

**Sequence similarities** Belongs to the Bcl-2 family.

**Domain** 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.

**Cellular localization** 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.

## Images



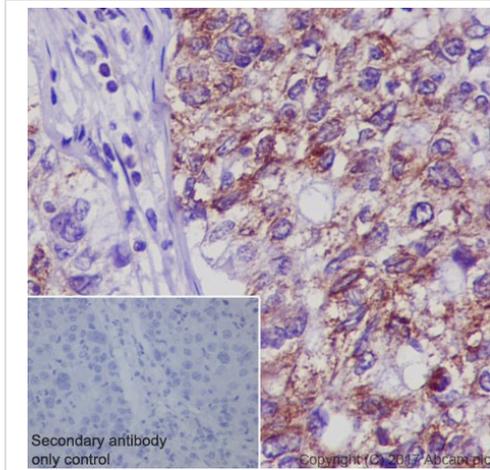
Western blot - Anti-Bax antibody [E63] (ab32503)

**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

**Lane 2:** Bax knockout HAP1 cell lysate (20 µg)

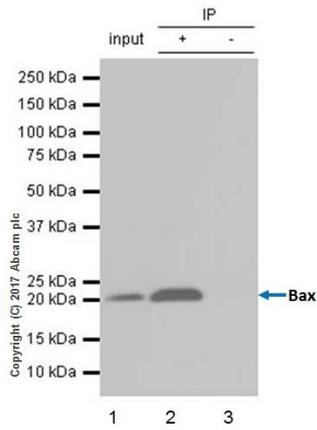
**Lanes 1 - 2:** Merged signal (red and green). Green - ab32503 observed at 20 kDa. Red - loading control, [ab8245](#), observed at 37 kDa or [ab18058](#), observed at 130 kDa.

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



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

Purified ab32503 staining Bax in Human lung carcinoma tissue section by immunohistochemistry (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.



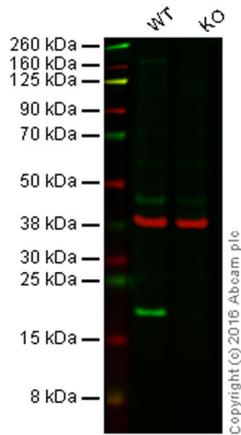
Immunoprecipitation - Anti-Bax antibody [E63] (ab32503)

**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 (HRP) was used as the secondary antibody at 1/1000 dilution. Capture antibody was used at a 1/20 dilution. Blocking and diluting buffer used was 5% NFDm/TBST.



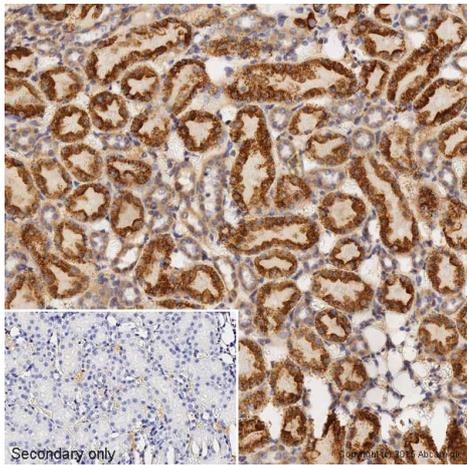
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**Lane 2:** Bax knockout HAP1 cell lysate (20 µg)

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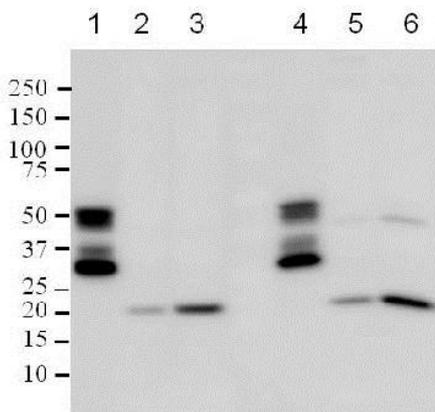
ab32503 was shown to recognize Bax when Bax knockout samples were used, along with additional cross-reactive bands. Wild-type and Bax knockout samples were subjected to SDS-PAGE. ab32503 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 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)

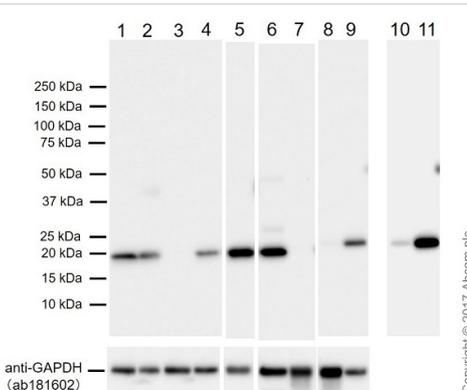
IHC image of ab32503 staining Bax in rat kidney formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with 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)

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)

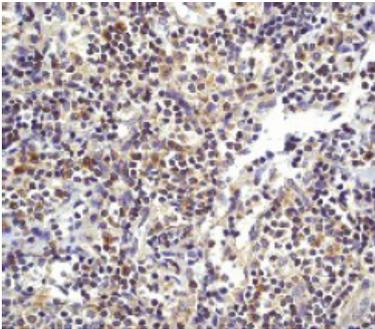
**Predicted band size:** 21 kDa

**Observed band size:** 18 kDa [why is the actual band size different from the predicted?](#)

Blocking and Diluting buffers: 5% NFDm/TBST

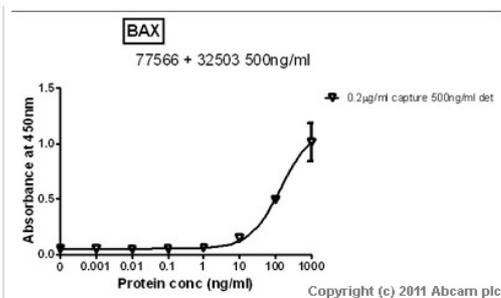
Exposure time 1~9 lanes 32 s; 10~11 lanes 3 min

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



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

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



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

Standard Curve for Bax (Analyte: [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.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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