abcam

ab32445

Anti-Bad antibody [Y208]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y208
Purification technique	Affinity purification Protein A
Specificity	This antibody does not cross-react with other Bcl-2 members.The mouse and rat recommendation is based on the IHC-P results. We do not guarantee WB for mouse and rat.
Concentration	0.145 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/1000

Notes	The mouse and rat recommendation is based on the IHC-P results. We do not guarantee WB for mouse and rat." in the "Specificity" section and "WB Application

Species	Rat
Dilution info	1/1000
Notes	The mouse and rat recommendation is based on the IHC-P results. We do not guarantee WB for mouse and rat." in the "Specificity" section and "WB Application

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Dog
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Dog

Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Dog
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20 - 1/50
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.

Notes	-

Predicted

Species	Dog
Dilution info	-
Notes	-

Target data

See full target information BAD

Function	Promotes cell death. Successfully competes for the binding to Bcl-X(L), Bcl-2 and Bcl-W, thereby affecting the level of heterodimerization of these proteins with BAX. Can reverse the death repressor activity of Bcl-X(L), but not that of Bcl-2 (By similarity). Appears to act as a link between growth factor receptor signaling and the apoptotic pathways.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Bad protein also known as Bcl-2-associated death promoter plays a critical role in apoptosis regulation. It possesses a molecular weight of approximately 23 kDa. Bad is expressed widely in tissues and is a member of the Bcl-2 family of proteins. This family is well-known for its involvement in the regulation of cell death both promoting and inhibiting depending on the protein's interactions.
Biological function summary	Its main role is to promote apoptosis by binding to anti-apoptotic proteins like Bcl-2 and Bcl-XL displacing pro-apoptotic Bax and Bak proteins to trigger cell death. Bad functions as part of the mitochondrial apoptosis pathway and is known to form complexes with phosphorylated proteins. Phosphorylation of Bad by kinases can lead to its sequestration to the cytoplasm reducing apoptosis.
Pathways	Its action is pivotal in the PI3K/Akt signaling pathway. This pathway involves growth factors and cell survival signals where phosphorylation of Bad inhibits its pro-apoptotic activity. Bad interacts with proteins like Akt and 14-3-3 to modulate cell survival. Disruption in its regulation through these pathways may lead to abnormal cell survival or death.
Associated diseases and disorders	Bad has implications in cancer and neurodegenerative diseases. Cancer cells often exhibit impaired apoptotic pathways where overexpression of Bad can ameliorate responsiveness to therapy. In neurodegenerative diseases such as Alzheimer's dysregulation of its function can contribute to cell death. The protein's interaction with Bcl-2 and Bcl-XL is significant in these contexts influencing disease propagation and severity.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

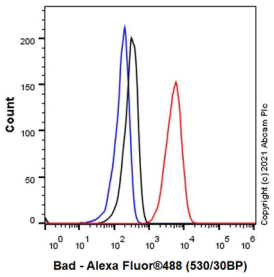
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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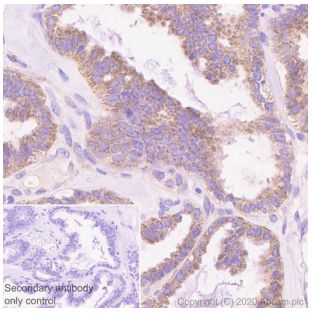
9 product images



Flow Cytometry (Intracellular) - Anti-Bad antibody [Y208] (ab32445)

Bad Flow Cytometry (Intracellular) staining using rabbit Anti-Bad antibody

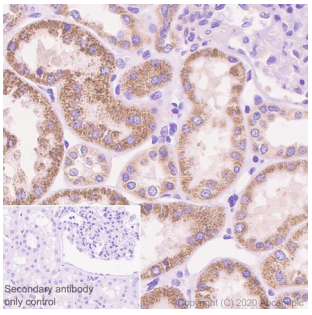
Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labelling Bad with purified ab32445 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

Bad Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bad antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian cancer tissue sections labeling Bad with purified ab32445 at 1/1000 dilution (0.14 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

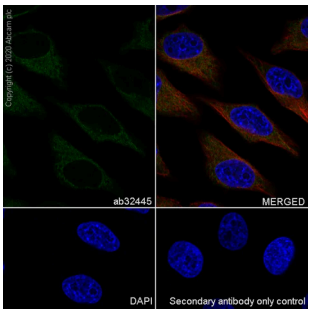


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

Bad Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bad antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling Bad with purified ab32445 at 1/1000 dilution (0.14 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used. PBS instead of the

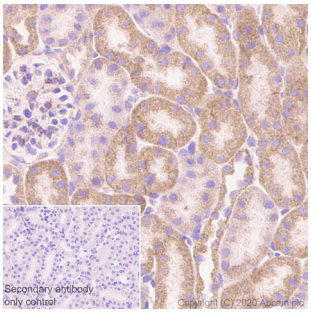
primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunocytochemistry/ Immunofluorescence - Anti-Bad antibody [Y208] (ab32445)

Bad Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bad antibody

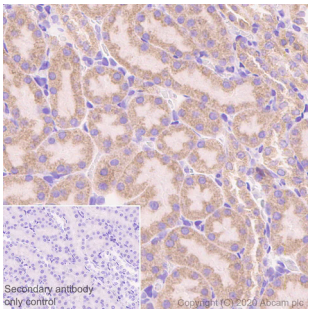
Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Bad with purified ab32445 at 1/50 dilution (2.9 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

Bad Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bad antibody

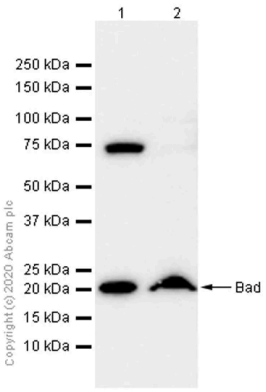
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Bad with purified ab32445 at 1/1000 dilution (0.14 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

Bad Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bad antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue sections labeling Bad with purified ab32445 at 1/1000 dilution (0.14 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-Bad antibody [Y208] (ab32445)

Bad Western blot staining using rabbit Anti-Bad antibody

All lanes:
Western blot - Anti-Bad antibody [Y208] (ab32445) at 1/2000 dilution

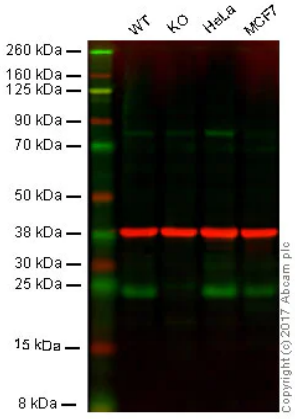
Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

Lane 2:
HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 18 kDa



Western blot - Anti-Bad antibody [Y208] (ab32445)

Bad Western blot staining using rabbit Anti-Bad antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab32445 observed at 23 kDa. Red - loading control, ab9484, observed at 37 kDa.
ab32445 was shown to specifically recognise BAD in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when BAD knockout cells were examined. Wild-type and BAD knockout samples were subjected to SDS-PAGE. ab32445 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Bad antibody [Y208] (ab32445) at 1/2000 dilution

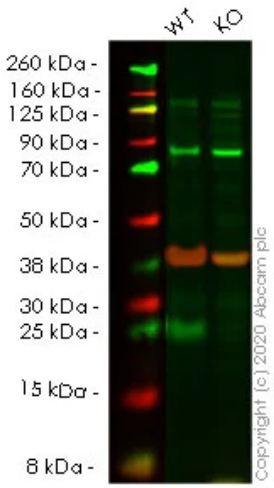
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
BAD knockout HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
MCF7 whole cell lysate at 20 µg

Predicted band size: 18 kDa



Western blot - Anti-Bad antibody [Y208] (ab32445)

Lanes 1- 2: Merged signal (red and green). Green - ab32445 observed at 23 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.
ab32445 was shown to react with Bad in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264843 (knockout cell lysate ab256847) was used. Wild-type HeLa and BAD 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. ab32445 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 2000 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.

All lanes:
Western blot - Anti-Bad antibody [Y208] (ab32445) at 1/2000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
BAD knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 18 kDa

Observed band size: 23 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.