abcam

Product datasheet

Anti-Bad antibody [Y208] ab32445

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Bad antibody [Y208]
Description	Rabbit monoclonal [Y208] to Bad
Host species	Rabbit
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.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Dog
Immunogen	Synthetic peptide within Human Bad (N terminal). The exact sequence is proprietary. (Peptide available as **ab206866**)
Positive control	ICC/IF: HeLa cells; IHC-P: Human ovarian cancer, Human, Mouse and Rat kidney tissue; Flow Cyt (intra): MCF7 cells. WB: HeLA and HepG2 whole cell lysate.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y208
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32445 in the following tested applications.

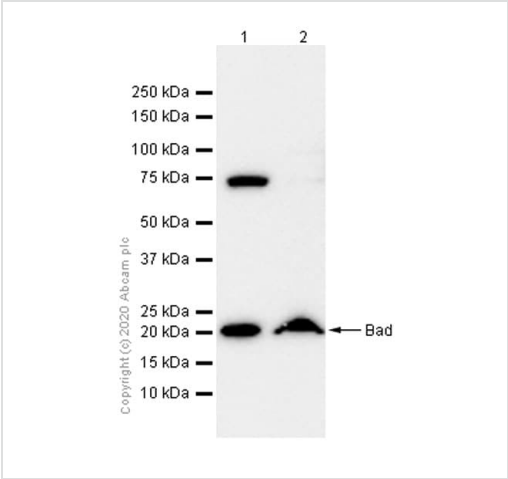
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20 - 1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (7)	1/1000. Detects a band of approximately 23 kDa (predicted molecular weight: 18 kDa).
IHC-P	★★★★☆ (1)	1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. 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
ICC/IF	★★★☆☆ (1)	1/50.

Target

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.
Tissue specificity	Expressed in a wide variety of tissues.
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.
Post-translational modifications	Phosphorylated on one or more of Ser-75, Ser-99, Ser-118 and Ser-134 in response to survival stimuli, which blocks its pro-apoptotic activity. Phosphorylation on Ser-99 or Ser-75 promotes heterodimerization with 14-3-3 proteins. This interaction then facilitates the phosphorylation at Ser-118, a site within the BH3 motif, leading to the release of Bcl-X(L) and the promotion of cell survival. Ser-99 is the major site of AKT/PKB phosphorylation, Ser-118 the major site of protein kinase A (CAPK) phosphorylation. Ser-75 is phosphorylated by AKT/PKB, protein kinase A and PIM2.
Cellular localization	Mitochondrion outer membrane. Cytoplasm. Upon phosphorylation, locates to the cytoplasm.

Images



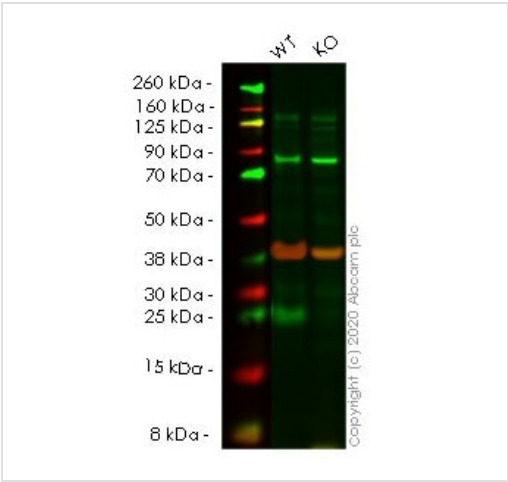
Western blot - Anti-Bad antibody [Y208] (ab32445)

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

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

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

Predicted band size: 18 kDa



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

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

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

Lysates/proteins at 20 µg per lane.

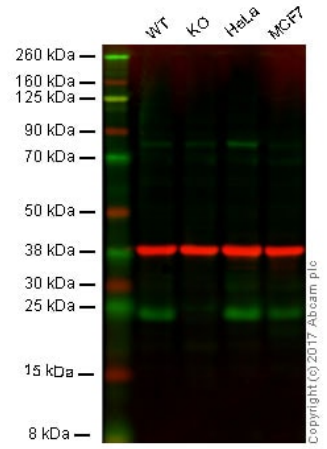
Performed under reducing conditions.

Predicted band size: 18 kDa
Observed band size: 23 kDa

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.



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

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

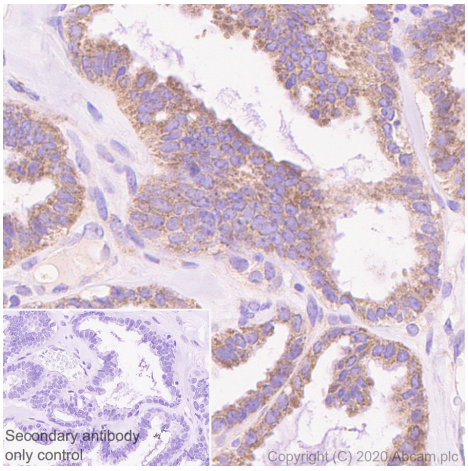
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : BAD knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 18 kDa

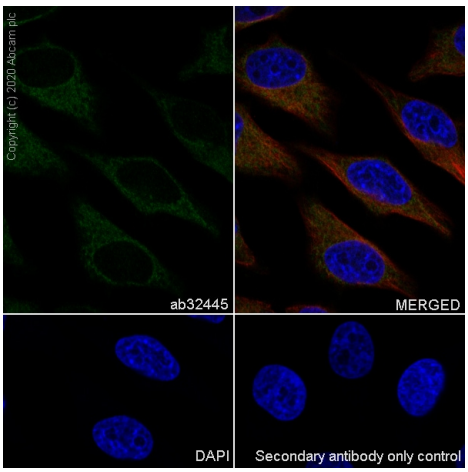
Lanes 1 - 4: Merged signal (red and green). Green - ab32445 observed at 23 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

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.



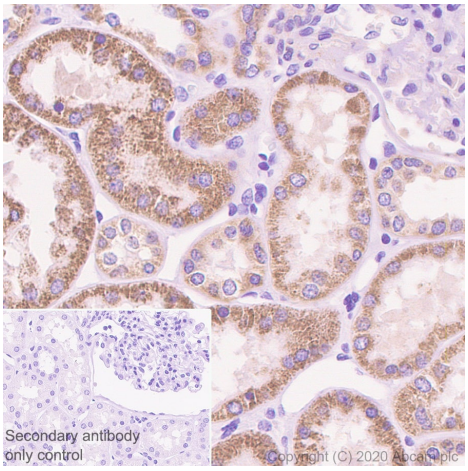
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

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 at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



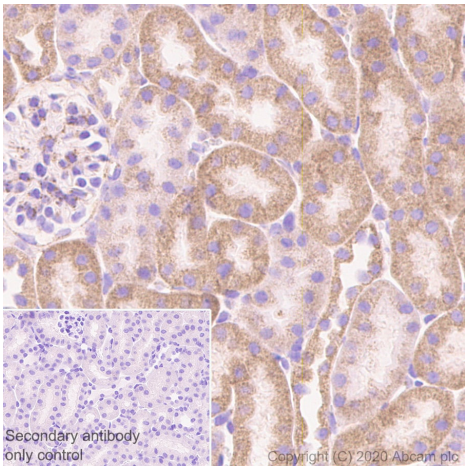
Immunocytochemistry/ Immunofluorescence - Anti-Bad antibody [Y208] (ab32445)

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.



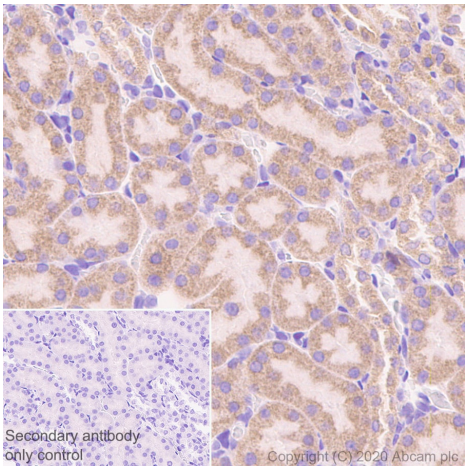
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

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 at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



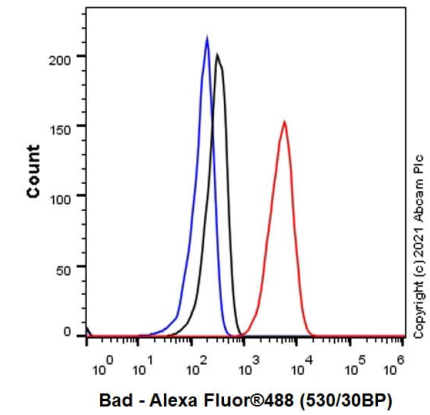
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

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 at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



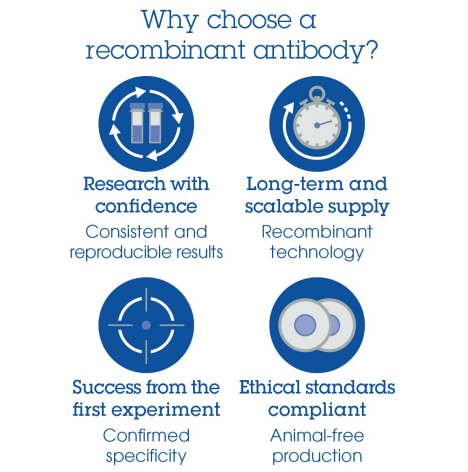
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bad antibody [Y208] (ab32445)

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 at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



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

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).



Anti-Bad antibody [Y208] (ab32445)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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