abcam

Product datasheet

Anti-Bak antibody [Y164] ab32371

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Bak抗体[Y164]
描述	兔单克隆抗体[Y164] to Bak
宿主	Rabbit
特异性	This antibody recognises Bak. The antibody does not cross-react with other Bcl2 members.
经测试应用	**适用于:** IP, Flow Cyt (Intra), WB, IHC-P **不适用于:** ICC/IF
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Recombinant Human Bak protein (**ab114337**), HAP1, HeLa and HEK-293 cell lysates; human heart and fetal heart lysates. IHC-P: Human pancreatic carcinoma, stomach carcinoma and normal stomach tissue. IP: HCT 116 and HeLa cell lysates. Flow Cyt (intra): HeLa cells.
常规说明	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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	Y164
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32371于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

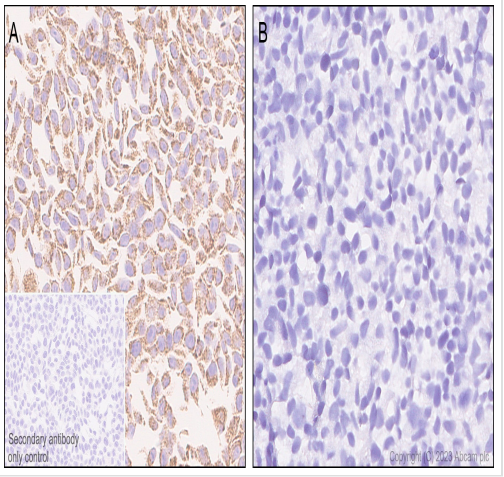
应用	Ab评论	说明
IP	★★★★★ (1)	Use at an assay dependent concentration.
Flow Cyt (Intra)		1/10 - 1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (1)	1/10000. Predicted molecular weight: 23 kDa. **For unpurified use at 1/1000 - 1/5000.**
IHC-P	★★★★★ (1)	1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

应用说明	Is unsuitable for ICC/IF.

靶标

功能	In the presence of an appropriate stimulus, accelerates programmed cell death by binding to, and antagonizing the anti-apoptotic action of BCL2 or its adenovirus homolog E1B 19k protein. Low micromolar levels of zinc ions inhibit the promotion of apoptosis.
组织特异性	Expressed in a wide variety of tissues, with highest levels in the heart and skeletal muscle.
序列相似性	Belongs to the Bcl-2 family.
结构域	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.
细胞定位	Mitochondrion membrane.

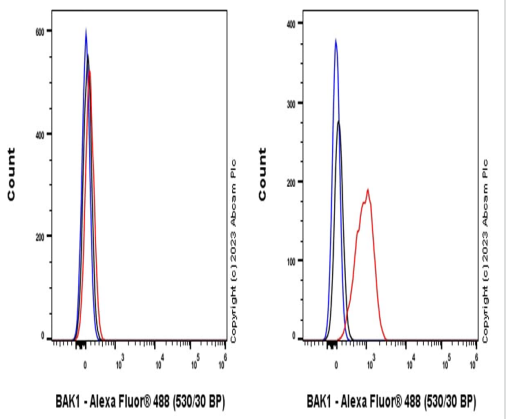
图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

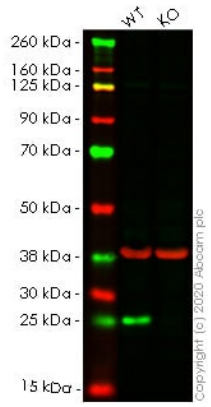
Immunohistochemical analysis of paraffin-embedded fixed (A) Wild-type HeLa (human cervix adenocarcinoma epithelial cell) cell pellet. (B) BAK1 knockout HeLa (**ab265277**) cell pellet staining Bak with ab32371 at 1/2000 dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Counter-staining used was hematoxylin.
Positive staining on (A) Wild-type HeLa cell pellet, no staining on (B) BAK1 knockout HeLa (**ab265277**) cell pellet.
The section was incubated with ab32371 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-Bak antibody [Y164] (ab32371)

Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized BAK1 KO Hela (human BAK1 knockout cervical adenocarcinoma epithelial cell, Left) /Parental Hela (Right) cells labelling Bak with ab32371 at 1/500 dilution (0.1 µg)(Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).
Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/5000 dilution was used as the secondary antibody.



Western blot - Anti-Bak antibody [Y164] (ab32371)

All lanes : Anti-Bak antibody [Y164] (ab32371) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BAK1 knockout HeLa cell lysate

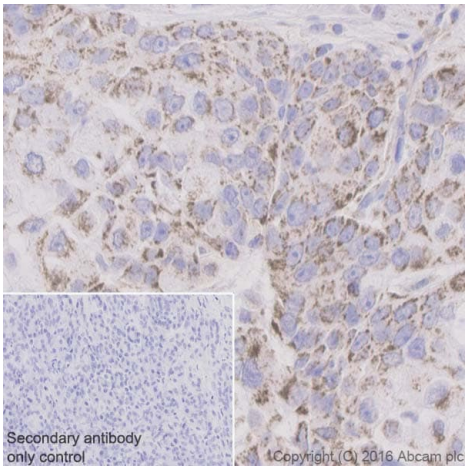
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

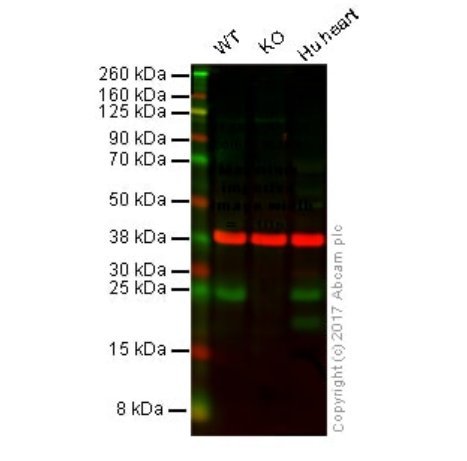
Lanes 1- 2: Merged signal (red and green). Green - ab32371 observed at 23 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32371 was shown to react with Bak in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265277** (knockout cell lysate **ab257077**) was used. Wild-type HeLa and BAK1 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. ab32371 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-Bak antibody [Y164] (ab32371)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human pancreatic carcinoma tissue sections labeling Bak with Purified ab32371 at 1:200 dilution (2.98 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. **ab97051** Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Bak antibody [Y164] (ab32371)

All lanes : Anti-Bak antibody [Y164] (ab32371)

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : BAK knockout HAP1 whole cell lysate
Lane 3 : Human Heart whole cell lysate

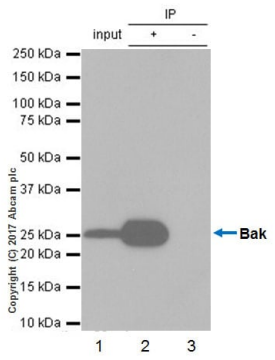
Lysates/proteins at 20 µg per lane.

Predicted band size: 23 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab32371 observed at 25 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

Unpurified ab32371 was shown to specifically recognize BAK in wild-type HAP1 cells. No band was observed when BAK knockout cells were examined. Wild-type and BAK knockout samples were subjected to SDS-PAGE. Unpurified ab32371 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.

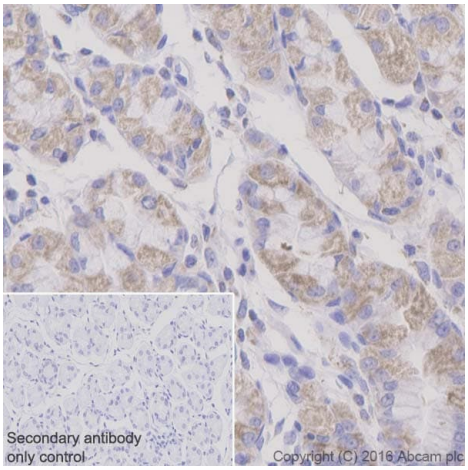


Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

ab32371 (purified) at 1:20 dilution (2µg) immunoprecipitating Bak in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

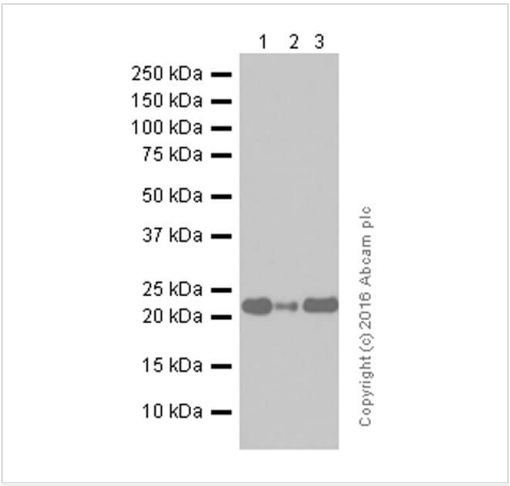
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32371 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32371 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach tissue sections labeling Bak with Purified ab32371 at 1:200 dilution (2.98 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. **ab97051** Goat Anti-Rabbit IgG H&L (HRP)
secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Bak antibody [Y164] (ab32371)

All lanes : Anti-Bak antibody [Y164] (ab32371) at 1/10000 dilution (purified)

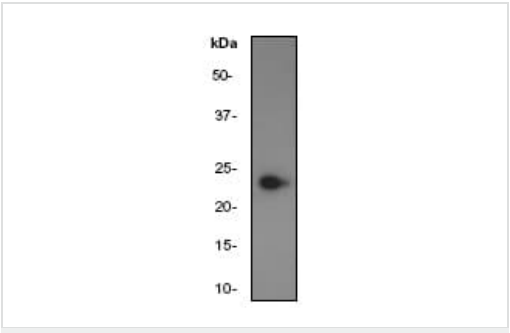
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates
Lane 3 : Human fetal heart lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

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

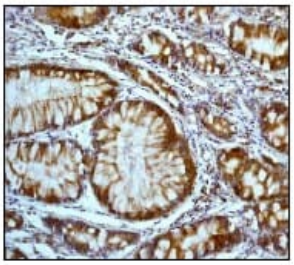
Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-Bak antibody [Y164] (ab32371)

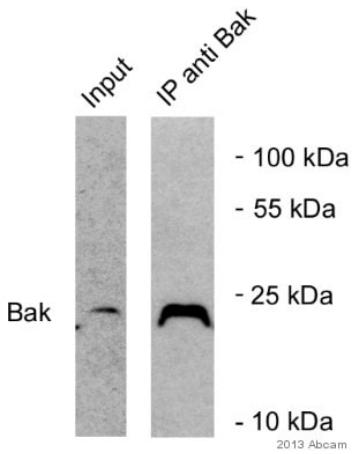
Anti-Bak antibody [Y164] (ab32371) at 1/5000 dilution (unpurified) + HeLa cell lysate

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

Immunohistochemical analysis of Bak expression in paraffin embedded human stomach carcinoma, using 1/250 unpurified ab32371.



Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

Bak was immunoprecipitated from HCT116 p53-/- cell line whole cell lysate with unpurified ab32371 at 1/100 dilution.

Western blot was performed from the immunoprecipitate using ab32371 at 1/2000 dilution.

Anti-Bak antibody [Y164] (ab32371)

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