abcam

ab10433

Anti-BNIP3 antibody [ANa40]

KO Validated

Key facts

Isotype	IgG2b
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	ANa40
Purification technique	Affinity purification
Epitope	The epitope recognized by the antibody resides within amino acids 112-124 of human BNIP3 molecule.
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	5 µg/mL
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	0.1-1 µg for 10^6 Cells
Notes	ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1-5 µg/mL
Notes	We recommend using 3% milk as the blocking agent for Western blot.

Predicted

Species	Mouse, Rat
Dilution info	-

Notes	-

Target data

See full target information BNIP3

Function	Apoptosis-inducing protein that can overcome BCL2 suppression. May play a role in repartitioning calcium between the two major intracellular calcium stores in association with BCL2. Involved in mitochondrial quality control via its interaction with SPATA18/MIEAP: in response to mitochondrial damage, participates in mitochondrial protein catabolic process (also named MALM) leading to the degradation of damaged proteins inside mitochondria. The physical interaction of SPATA18/MIEAP, BNIP3 and BNIP3L/NIX at the mitochondrial outer membrane regulates the opening of a pore in the mitochondrial double membrane in order to mediate the translocation of lysosomal proteins from the cytoplasm to the mitochondrial matrix. Plays an important role in the calprotectin (S100A8/A9)-induced cell death pathway.

Storage

Shipped at conditions	Blue Ice
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

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

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.

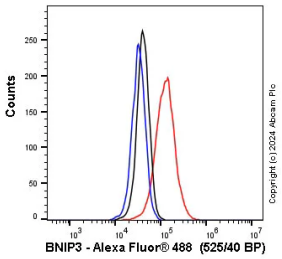
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

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5 product images



Flow Cytometry (Intracellular) - Anti-BNIP3 antibody [ANa40] (ab10433)

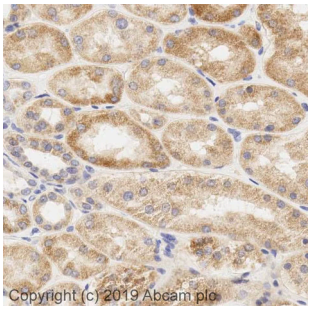
BNIP3 Flow Cytometry (Intracellular) staining of HepG2 cells using mouse Anti-BNIP3 antibody

Flow cytometry overlay histogram showing HepG2 cells stained with ab10433 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab10433) ($1x10^6$ in 100µl at 5.0µg/ml) for 30 min at 22°C.

The secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (ab150117) preadsorbed was incubated at 1/4000 for 30 min at 22°C.

Isotype control antibody (black line) was Mouse IgG2b, kappa monoclonal [7E10G10] (ab170192) used under the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BNIP3 antibody [ANa40] (ab10433)

BNIP3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-BNIP3 antibody

IHC image of BNIP3 antibody staining in a section of formalin-fixed paraffin-embedded normal human kidney* performed on a Leica BOND^{TM} system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab10433, 1ug/ml, 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.
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.

** Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*

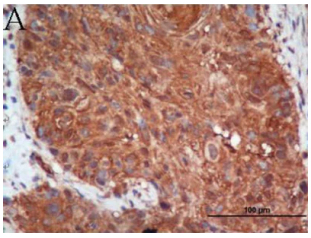
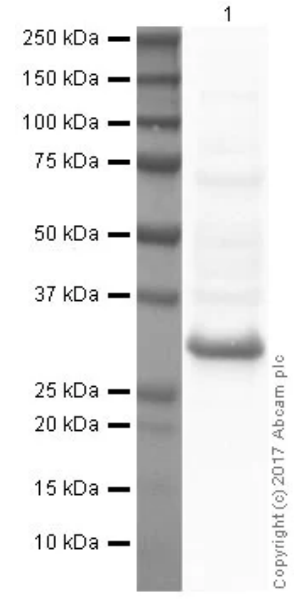


Image from Jin T et al., J Transl Med. 2012 Mar 30;10:64 Fig 1.; doi:10.1186/1479-5876-10-64; 30 March 2012, Journal of Translational Medicine 2012, 10:64

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BNIP3 antibody [ANa40] (ab10433)

Immunohistochemical analysis of Human laryngeal squamous cell carcinoma tissue, staining BNIP3 with ab10433.

Antigen retrieval was performed by heat mediation in citrate buffer. The sections were incubated with primary antibody (1/100) overnight at 4°C in a humidified chamber. Staining was visualized using DAB, followed by hematoxylin nuclear counterstaining.



Western blot - Anti-BNIP3 antibody [ANa40] (ab10433)

BNIP3 Western blot staining of Human skeletal muscle tissue lysate - total protein (ab29330) using mouse Anti-BNIP3 antibody

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab10433 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution ab133406.
All lanes:
Western blot - Anti-BNIP3 antibody [ANa40] (ab10433) at 1 µg/mL

All lanes:
Human skeletal muscle tissue lysate - total protein (ab29330) at 20 µg

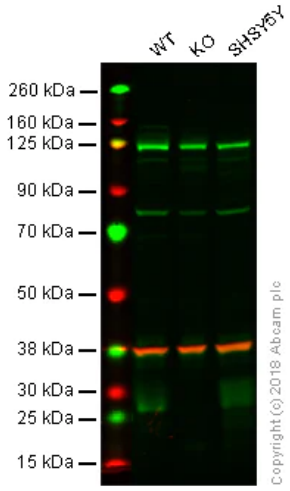
Secondary

All lanes:
Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) (ab65485) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 21 kDa

Exposure time: 5s



Western blot - Anti-BNIP3 antibody [ANa40] (ab10433)

BNIP3 Western blot staining using mouse Anti-BNIP3 antibody

Lane 1: Wild-type HAP1 whole cell lysate (40 µg)
Lane 2: BNIP3 knockout HAP1 whole cell lysate (40 µg)
Lane 3: SHSY5Y whole cell lysate (40 µg)
Lanes 1 - 3: Merged signal (red and green). Green - ab10433 observed at 30 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab10433 was shown to recognize BNIP3 in wild-type HAP1 cells as signal was lost at the expected MW in BNIP3 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and BNIP3 knockout samples were subjected to SDS-PAGE. ab10433 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 5 µg/mL and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-BNIP3 antibody [ANa40] (ab10433)

Predicted band size: 21 kDa

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