## Overview

### Product name
Anti-BNIP3 antibody [ANa40] ab10433

### Description
Mouse monoclonal [ANa40] to BNIP3

### Host species
Mouse

### Specificity
This antibody was originally tested on whole cell lysate from 293T cells transfected with BNIP3. Levels of constitutively expressed BNIP3 are very variable and a good positive control is strongly recommended. Zhang et al. (2008) comment "Very low levels of BNIP3 protein were detected by immunoblot assay of lysates prepared from WT MEFs that were cultured at 20% O2, whereas hypoxia strongly induced expression of BNIP3 protein, which migrated as a 30-kDa monomer and 60-kDa dimer...BNIP3 expression is regulated by HIF-1 under physiological conditions in vivo."

### Tested applications
**Suitable for:** ICC, ELISA, IP, WB, IHC-P, Flow Cyt, ICC/IF

### Species reactivity
**Reacts with:** Mouse, Rat, Human

### Immunogen
Recombinant fragment (Residues 1-163 of Human BNIP3).

### Epitope
The epitope recognized by the antibody resides within amino acids 112-124 of human BNIP3 molecule.

### Positive control
This antibody gave a positive signal in human skeletal muscle tissue lysate in western blot and on human kidney formalin-fixed, paraffin-embedded tissue sections in IHC.

### General notes
**Western blot protocol advice:**
We recommend using 3% milk as the blocking agent for Western blot.

This antibody clone is manufactured by Abcam.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information [here](#).

## 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 or -80°C. Avoid freeze / thaw cycle.

### Storage buffer
pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

Purity        Protein G purified
Clonality     Monoclonal
Clone number  ANa40
Myeloma       unknown
Isotype       IgG2b
Light chain type  kappa

Applications

Our Abpromise guarantee covers the use of ab10433 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 30 kDa (predicted molecular weight: 30 kDa). We recommend using 3% milk as the blocking agent for Western blot.</td>
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<tr>
<td>IHC-P</td>
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<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 0.1-1µg for 10⁶ cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Target

Function        Apoptosis-inducing protein that, which can overcome BCL2 suppression. May play a role in repartitioning calcium between the two major intracellular calcium stores in association with BCL2.
Sequence similarities Belongs to the NIP3 family.

Images
**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.

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.
Anti-BNIP3 antibody [ANa40] (ab10433) at 1 µg/ml + Human skeletal muscle tissue lysate - total protein (ab29330) at 20 µg

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

Performed under reducing conditions.

**Predicted band size**: 30 kDa

**Exposure time**: 5 seconds

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.
IHC image of ab10433 staining in human normal kidney formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. 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 ab10433, 5µg/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.

ab10433 staining BNIP3 in Human fibroblasts by Immunocytochemistry/ Immunofluorescence. Cells were fixed in 2% paraformaldehyde for 30 minutes at room temperature and cells were treated with 50 mM NH4Cl in PBS for 10 minutes to quench free aldehyde groups after permeabilisation. Cells were then permeabilized in 0.1% Triton X-100 prior to blocking in 1% BSA for 1 hour at room temperature. The primary antibody was diluted 1/400 and incubated with the sample for 1 hour at room temperature. The secondary antibody was Alexa Fluor® 555-conjugated goat anti-mouse polyclonal, diluted 1/1000.
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-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab10433, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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