

# BNIP3 Antibody (BH3 Domain Specific)

Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP1321a

### **Product Information**

Application	IF, WB, IHC-P, E
Primary Accession	<u>Q12983</u>
Reactivity	Human, Mouse
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	21541
Antigen Region	215-252

## **Additional Information**

Gene ID	664
Other Names	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3, BNIP3, NIP3
Target/Specificity	This BNIP3 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 215-252 amino acids from human BNIP3.
Dilution	IF~~1:50~100 WB~~1:1000 IHC-P~~1:100~500 E~~Use at an assay dependent concentration.
Format	Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is prepared by Saturated Ammonium Sulfate (SAS) precipitation followed by dialysis against PBS.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Precautions	BNIP3 Antibody (BH3 Domain Specific) is for research use only and not for use in diagnostic or therapeutic procedures.

### **Protein Information**

Name	BNIP3 ( <u>HGNC:1084</u> )
Synonyms	NIP3
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.
Cellular Location	Mitochondrion. Mitochondrion outer membrane; Single-pass membrane protein. Note=Coexpression with the EIB 19-kDa protein results in a shift in NIP3 localization pattern to the nuclear envelope. Colocalizes with ACAA2 in the mitochondria. Colocalizes with SPATA18 at the mitochondrion outer membrane

### Background

NIP3 is a member of the BCL2/adenovirus E1B 19 kd-interacting protein (BNIP) family. It interacts with the E1B 19 kDa protein which is responsible for the protection of virally-induced cell death, as well as E1B 19 kDa-like sequences of BCL2, also an apoptotic protector. NIP3 contains a BH3 domain and a transmembrane domain, which have been associated with pro-apoptotic function. The dimeric mitochondrial protein is known to induce apoptosis, even in the presence of BCL2.

### References

References for protein:

Kothari, S., et al., Oncogene 22(30):4734-4744 (2003).
Lee, S.M., et al., Life Sci. 71(19):2267-2277 (2002).
Ray, R., et al., J. Biol. Chem. 275(2):1439-1448 (2000).
Chen, G., et al., J. Biol. Chem. 274(1):7-10 (1999).
Yasuda, M., et al., J. Biol. Chem. 273(20):12415-12421 (1998).

References for HepG2 cell line:

1. Knowles BB, et al. (1980). Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science 209: 497-499.[PubMed: 6248960].

2. Darlington GJ, et al. (1987). Growth and hepatospecific gene expression of human hepatoma cells in a defined medium. In Vitro Cell. Dev. Biol. 23: 349-354. [PubMed: 3034851].

3. Ihrke, G; Neufeld, EB; Meads, T; Shanks, MR; Cassio, D; Laurent, M; Schroer, TA; Pagano, RE et al. (1993). "WIF-B cells: an in vitro model for studies of hepatocyte polarity". Journal of Cell Biology 123 (6): 1761–1775. [PubMed:7506266].

4. Mersch-Sundermann, V.; Knasmüller, S.; Wu, X. J.; Darroudi, F.; Kassie, F. (2004). "Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents". Toxicology 198 (1–3): 329–340. [PubMed:15138059].

#### Images

Fluorescent confocal image of HepG2 cells stained with BNIP3 (BH3 Domain Specific) antibody. HepG2 cells were fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP1321a BNIP3 (BH3 Domain Specific) primary antibody (1:500, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Nuclei were counterstained with Hoechst 33342 (blue) (10 µg/ml, 5



1 2

150

100 75

> 50 37

> 25 20 15

min). BNIP3 immunoreactivity is localized to the cytoplasm of HepG2 cells.

The anti-NIP3 BH3 domain Pab (Cat. #AP1321a) is used in Western blot to detect NIP3 BH3 in Ramos cell lysate (lane 1) and in mouse brain tissue lysate (lane 2).



Freshly isolated mouse hepatocytes plated on coverslips (2 x105 cells/22-mm glass coverslip) were cultured under normoxic conditions for 6 hr. The cells were then fixed in 2% paraformaldehyde in PBS for 1 hr, and processed for confocal immunofluorescence (red: F-actin, blue: ATP-synthase, green: BNIP3). Fluorescence labeling of BNIP3 accomplished with anti-BNIP3 antibody Cat # AP1321a. Data courtesy of Ruben Zamora, University of Pittsburgh.



Formalin-fixed and paraffin-embedded human cancer tissue reacted with the primary antibody, which was peroxidase-conjugated to the secondary antibody, followed by DAB staining. This data demonstrates the use of this antibody for immunohistochemistry; clinical relevance has not been evaluated. BC = breast carcinoma; HC = hepatocarcinoma.

## Citations

- Autophagy and Bcl-2/BNIP3 death regulatory pathway in non-small cell lung carcinomas.
- Chronic autophagy is a cellular adaptation to tumor acidic pH microenvironments.
- Expression and subcellular localization of BNIP3 in hypoxic hepatocytes and liver stress.

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