Anti-BNIP3 Antibody [JA71-10]

ET1704-08

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	JA71-10

Description: The adenovirus E1B protein is a viral homolog of the Bcl-2 family of proteins that are involved in regulating cell death. A family of interacting proteins, which are designated Nip or Bnip and include BNIP-1, BNIP-2, BNIP-3 and Nix, associate with both the E1B protein and Bcl-2 proteins to mediate apoptotic signaling. BNIP-1 contains a hydrophobic transmembrane domain, which enables its localization to the nuclear envelope, endoplasmic recticulum and mitochondria. BNIP-2, (previously designated Nip2 and Nip21 in human and mouse respectively), shares homology with the non-catalytic domain of Cdc42 GTPase-activating protein (Cdc42GAP). Through binding to Cdc42GAP, BNIP-2 enhances the GTPase activity of Cdc42GAP, facilitating the hydrolysis of GTP bound to Cdc42 and thereby, mediating the signaling pathways involving receptor kinases, small GTPases and apoptotic proteins. Nix, which is also designated Nip3L or Bnip3L, is highly related to BNIP-3, and both proteins localize to the mitochondria where they associate with Bcl-2 proteins. BNIP-3 preferentially binds to Bcl-xL and induces apoptosis by suppressing the anti-apoptosis activity of Bcl-xL.

Immunogen:	Synthetic peptide within human BNIP3 aa 90-130.
Positive control:	Mouse kidney tissue lysates, Hela, SH-SY5Y, NIH/3T3, human kidney tissue, mouse brain tissue, mouse kidney tissue, rat brain tissue, rat kidney tissue.
Subcellular location:	Membrane, Mitochondrion, Mitochondrion outer membrane.
Database links:	SwissProt: Q12983 Human | O55003 Mouse Entrez Gene: 84480 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:1,000

Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

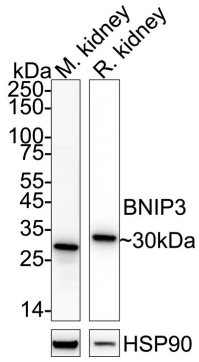

Fig1: Western blot analysis of BNIP3 on different lysates with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

Lane 1: Mouse kidney tissue lysate (40 µg/Lane)
Lane 2: Rat kidney tissue lysate (40 µg/Lane)

Predicted band size: 22 kDa
Observed band size: 30 kDa

Exposure time: Lane 1: 1 minute 2 seconds; Lane 2: 8 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-08) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

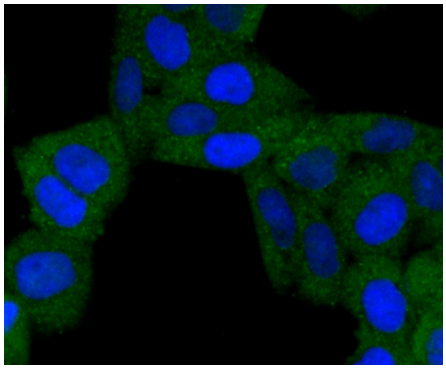

Fig2: ICC staining of BNIP3 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-08, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

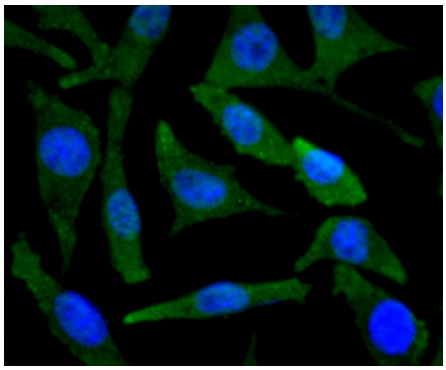

Fig3: ICC staining of BNIP3 in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-08, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

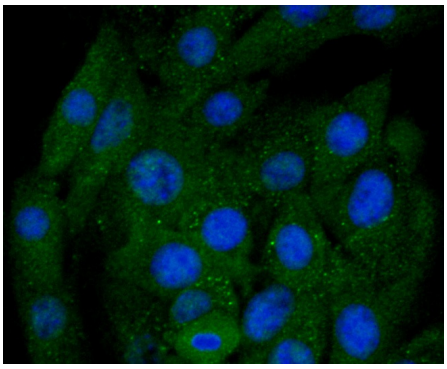

Fig4: ICC staining of BNIP3 in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-08, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

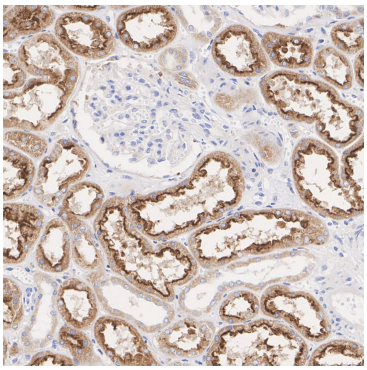

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-08) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

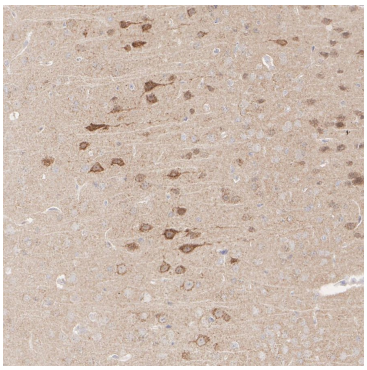

Fig6: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-08) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

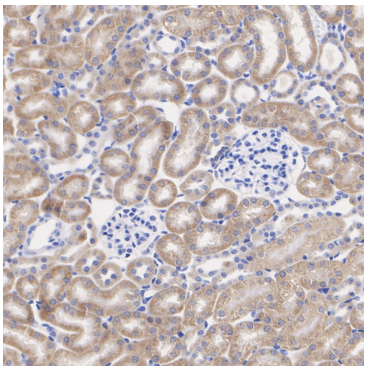

Fig7: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-08) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

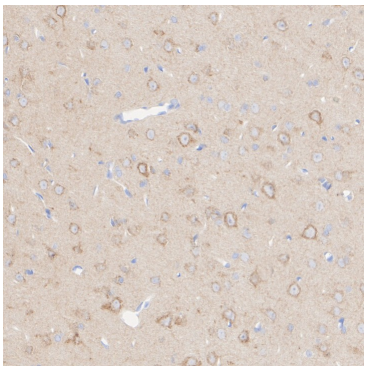

Fig8: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-08) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

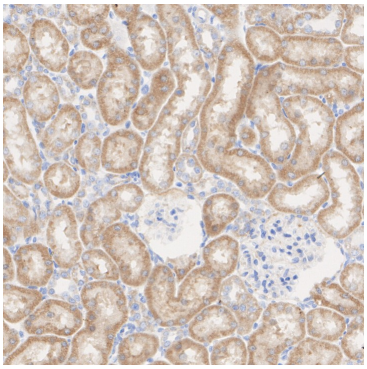

Fig9: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-BNIP3 antibody (ET1704-08) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-08) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Ono M et al. Enhanced snoMEN Vectors Facilitate Establishment of GFP-HIF-1a Protein Replacement Human Cell Lines. PLoS One 11:e0154759 (2016).
2. Fedorova LV et al. Peroxisome proliferator-activated receptor d agonist, HPP593, prevents renal necrosis under chronic ischemia. PLoS One 8:e64436 (2013).