Anti-Bax Antibody [SZ3-07]

ET1603-34

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Goat
Applications:	WB, IP, FC, IF-Cell
Molecular Wt:	Predicted band size: 21 kDa
Clone number:	SZ3-07

Description: In healthy mammalian cells, the majority of BAX is found in the cytosol, but upon initiation of apoptotic signaling, Bax undergoes a conformational shift. Upon induction of apoptosis, BAX becomes organelle membrane-associated, and in particular, mitochondrial membrane associated. BAX is believed to interact with, and induce the opening of the mitochondrial voltage-dependent anion channel, VDAC. Alternatively, growing evidence also suggests that activated BAX and/or Bak form an oligomeric pore, MAC in the MOM (mitochondrial outer membrane). This results in the release of cytochrome c and other pro-apoptotic factors from the mitochondria, often referred to as mitochondrial outer membrane permeabilization, leading to activation of caspases. This defines a direct role for BAX in mitochondrial outer membrane permeabilization. BAX activation is stimulated by various abiotic factors, including heat, hydrogen peroxide, low or high pH, and mitochondrial membrane remodeling. In addition, it can become activated by binding BCL-2, as well as non-BCL-2 proteins such as p53 and Bif-1. Conversely, BAX can become inactivated by interacting with VDAC2, Pin1, and IBRDC2.

Immunogen: Synthetic peptide within Human Bax aa 1-50 / 192.

Positive control: HeLa cell lysate, MCF7 cell lysate, HEK-293 cell lysate, bEnd.3 cell lysate, C2C12 cell lysate, PC-12 cell lysate, C6 cell lysate, C2C12, HeLa.

Subcellular location: Mitochondrion membrane, Cytoplasm.

Database links: SwissProt: Q07812 Human | Q07813 Mouse | Q63690 Rat

Recommended Dilutions:

WB	1:20,000
FC	1:1,000
IP	Use at an assay dependent concentration.
IF-Cell	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

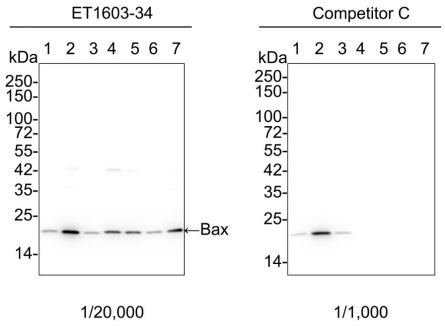

Fig1: Western blot analysis of Bax on different lysates with Rabbit anti-Bax antibody (ET1603-34) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: bEnd.3 cell lysate
Lane 5: C2C12 cell lysate
Lane 6: PC-12 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 30 seconds;

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 (ET1603-34) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

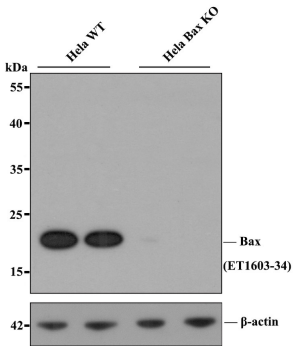

Fig2: All lanes: Western blot analysis of Bax with anti-Bax antibody [SZ3-07] (ET1603-34) at 1/1,000 dilution.
Lane 1/2: Wild-type Hela whole cell lysate (20 µg).
Lane 3/4: Bax knockout Hela whole cell lysate (20 µg).

ET1603-34 was shown to specifically react with Bax in wild-type Hela cells. No band was observed when Bax knockout samples were tested. Wild-type and Bax knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1603-34, 1/1,000) and Loading control antibody (Rabbit anti-β-actin, R1207-1, 1/1,000)was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

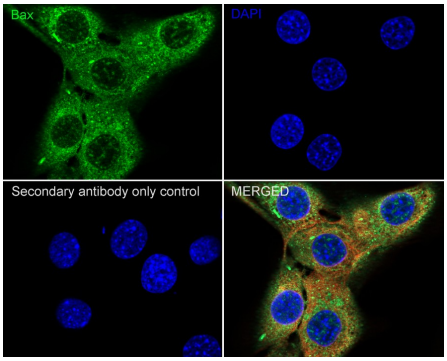

Fig3: Immunocytochemistry analysis of C2C12 cells labeling Bax with Rabbit anti-Bax antibody (ET1603-34) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Bax antibody (ET1603-34) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

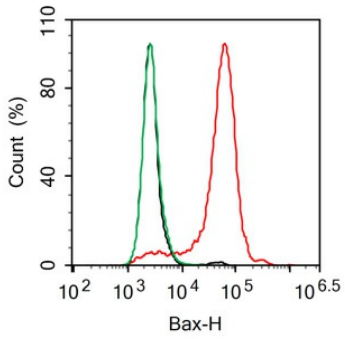

Fig4: Flow cytometric analysis of HeLa cells labeling Bax.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-34, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. He G et al. Gadd45b prevents autophagy and apoptosis against rat cerebral neuron oxygen-glucose deprivation/reperfusion injury. Apoptosis 21:390-403 (2016).
2. Chen B et al. Inhibition of miR-29c promotes proliferation, and inhibits apoptosis and differentiation in P19 embryonic carcinoma cells. Mol Med Rep 13:2527-35 (2016).