Anti-Cytochrome C Antibody [SC69-08]

ET1610-60

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Tissue, IHC-P, IP, IF-Cell
Molecular Wt:	Predicted band size: 12 kDa
Clone number:	SC69-08

Description: Cytochrome c is a well characterized mobile electron transport protein that is essential to energy conversion in all aerobic organisms. In mammalian cells, this highly conserved protein is normally localized to the mitochondrial intermembrane space. More recent studies have identifed cytosolic cytochrome c as a factor necessary for activation of apoptosis. During apoptosis, cytochrome c is translocated from the mitochondrial membrane to the cytosol, where it is required for activation of caspase-3 (CPP32). Overexpression of Bcl-2 has been shown to prevent the translocation of cytochrome c, thereby blocking the apoptotic process. Overexpression of Bax has been shown to induce the release of cytochrome c and to induce cell death. The release of cytochrome c from the mitochondria is thought to trigger an apoptotic cascade, whereby Apaf-1 binds to Apaf-3 (caspase-9) in a cytochrome c-dependent manner, leading to caspase-9 cleavage of caspase-3.

Immunogen: Synthetic peptide within human Cytochrome C aa 20-60.

Positive control: HeLa cell lysate, C6 cell lysate, Mouse brain tissue lysate, Mouse kidney tissue lysate, Rat brain tissue lysate, Rat kidney tissue lysate, Jurkat cell lysate, Human kidney tissue lysate, C6, human kidney tissue, mouse liver tissue, mouse kidney tissue, rat brain tissue.

Subcellular location: Mitochondrion intermembrane space.

Database links: SwissProt: P99999 Human | P62897 Mouse | P62898 Rat

Recommended Dilutions:

WB	1:2,000
IF-Tissue	1:50-1:100
IHC-P	1:1,000-1:5,000
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: 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

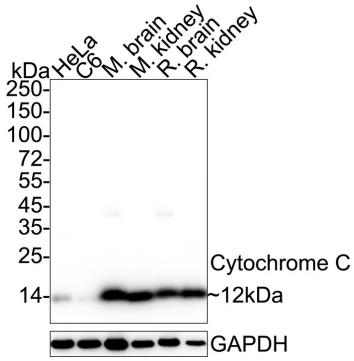

Fig1: Western blot analysis of Cytochrome C on different lysates with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: C6 cell lysate (10 µg/Lane)
Lane 3: Mouse brain tissue lysate (20 µg/Lane)
Lane 4: Mouse kidney tissue lysate (20 µg/Lane)
Lane 5: Rat brain tissue lysate (20 µg/Lane)
Lane 6: Rat kidney tissue lysate (20 µg/Lane)

Predicted band size: 12 kDa
Observed band size: 12 kDa

Exposure time: 4 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 (ET1610-60) at 1/2,000 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.

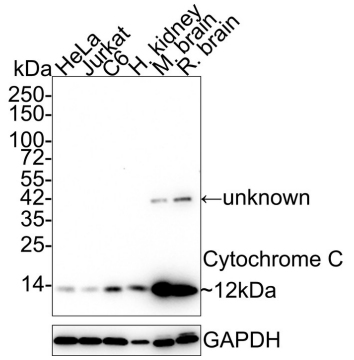

Fig2: Western blot analysis of Cytochrome C on different lysates with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Jurkat cell lysate (15 µg/Lane)
Lane 3: C6 cell lysate (15 µg/Lane)
Lane 4: Human kidney tissue lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (20 µg/Lane)
Lane 6: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 12 kDa
Observed band size: 12 kDa

Exposure time: 3 minutes; 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 (ET1610-60) at 1/2,000 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.

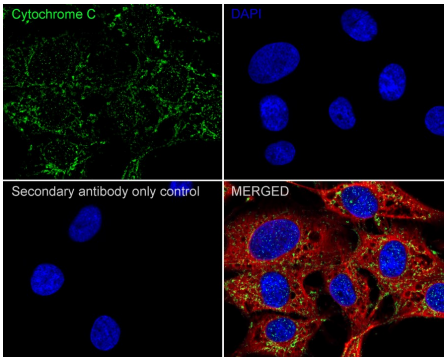

Fig3: Immunocytochemistry analysis of C6 cells labeling Cytochrome C with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/100 dilution.

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-Cytochrome C antibody (ET1610-60) 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.

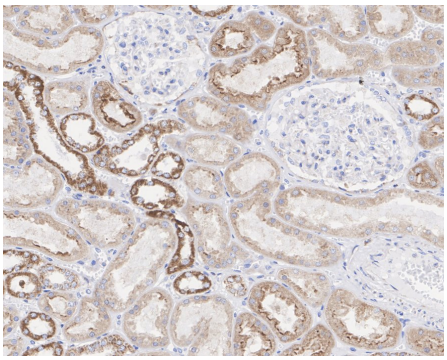

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/5,000 dilution.

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 (ET1610-60) at 1/5,000 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.

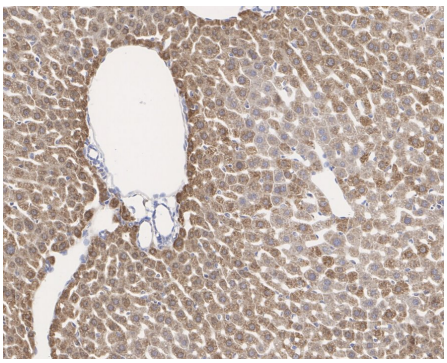

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/5,000 dilution.

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 (ET1610-60) at 1/5,000 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.

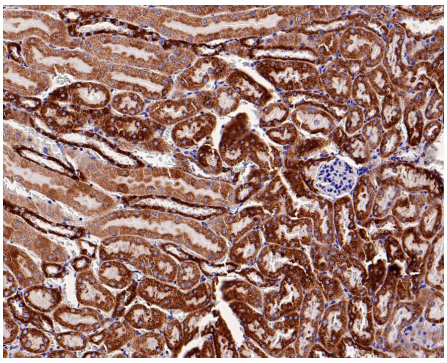

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/1,000 dilution.

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 (ET1610-60) 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.

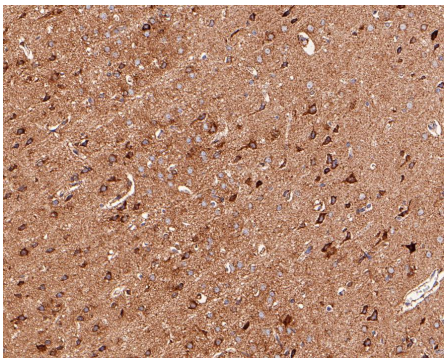

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Cytochrome C antibody (ET1610-60) at 1/1,000 dilution.

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 (ET1610-60) 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. Liu Z et al. Mark4 promotes oxidative stress and inflammation via binding to PPAR and activating NF-kB pathway in mice adipocytes. Sci Rep 6:21382 (2016).
2. Qi Y et al. PGC-1a Silencing Compounds the Perturbation of Mitochondrial Function Caused by Mutant SOD1 in Skeletal Muscle of ALS Mouse Model. Front Aging Neurosci 7:204 (2015).