Anti-VDAC1 Antibody [SA93-03]

ET1601-20

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	SA93-03

Description: Voltage-dependent anion-selective channel 1 (VDAC-1) is a beta barrel protein that in humans is encoded by the VDAC1 gene located on chromosome 5. It forms an ion channel in the outer mitochondrial membrane (OMM) and also the outer cell membrane. In the OMM, it allows ATP to diffuse out of the mitochondria into the cytoplasm. In the cell membrane, it is involved in volume regulation. Within all eukaryotic cells, mitochondria are responsible for synthesis of ATP among other metabolite needed for cell survival. VDAC1 therefore allows for communication between the mitochondrion and the cell mediating the balance between cell metabolism and cell death. Besides metabolic permeation, VDAC1 also acts as a scaffold for proteins such as hexokinase that can in turn regulate metabolism.

Immunogen: Synthetic peptide within N-terminal human VDAC1.

Positive control: MDA-MB-231 cell lysate, HeLa cell lysate, K-562 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse kidney tissue lysate, HeLa, HepG2, RH-35, human kidney tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Mitochondrion outer membrane, Cell membrane, Membrane raft

Database links: SwissProt: P21796 Human | Q60932 Mouse | Q9Z2L0 Rat

Recommended Dilutions:

WB	1:10,000
IF-Cell	1:50-1:100
IHC-P	1:1,000
FC	1:500-1:1,000
IF-Tissue	1:200

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

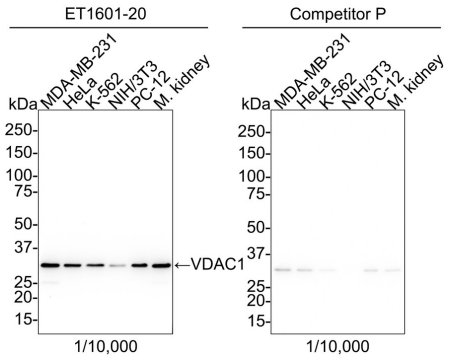

Fig1: Western blot analysis of VDAC1 on different lysates with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution.

Lane 1: MDA-MB-231 cell lysate (15 µg/Lane)
Lane 2: HeLa cell lysate (15 µg/Lane)
Lane 3: K-562 cell lysate (15 µg/Lane)
Lane 4: NIH/3T3 cell lysate (15 µg/Lane)
Lane 5: PC-12 cell lysate (15 µg/Lane)
Lane 6: Mouse kidney tissue lysate (15 µg/Lane)

Predicted band size: 31 kDa
Observed band size: 31 kDa
Exposure time: 14 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 (ET1601-20) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution were used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

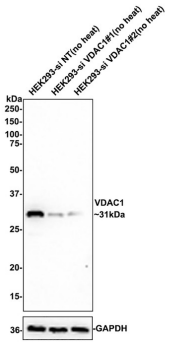

Fig2: Western blot analysis of VDAC1 on different lysates with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/1,000 dilution.

Lane 1: HEK293-si NT cell lysate (no heat) (10 µg/Lane)
Lane 2/3: HEK293-si VDAC1 cell lysate (no heat) (10 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 31 kDa
Observed band size: 31 kDa
Exposure time: 17 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

ET1601-20 was shown to specifically react with VDAC1 in HEK293-si NT cells. Weakened band were observed when HEK293-si VDAC1 samples were tested. HEK293-si NT and HEK293-si VDAC1 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 (ET1601-20, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at 4 ℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

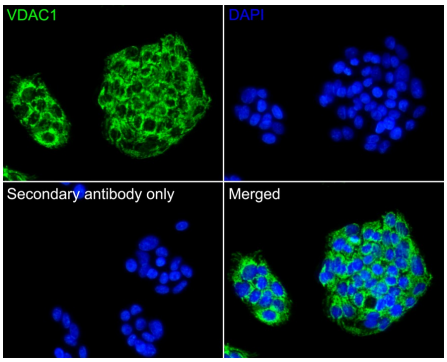

Fig3: Immunocytochemistry analysis of HepG2 cells labeling VDAC1 with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/50 dilution in 2% negative goat serum 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.

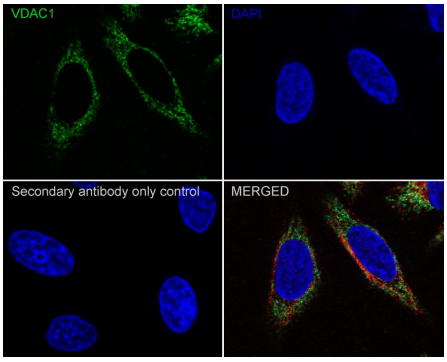

Fig4: Immunocytochemistry analysis of HeLa cells labeling VDAC1 with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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-VDAC1 antibody (ET1601-20) 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.

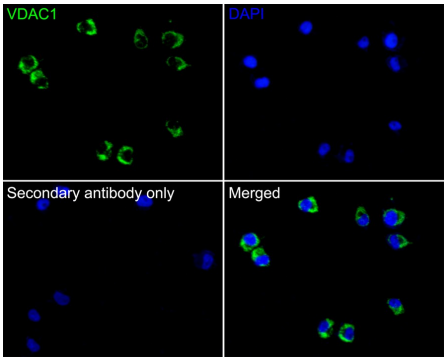

Fig5: Immunocytochemistry analysis of RH-35 cells labeling VDAC1 with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/50 dilution in 2% negative goat serum 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.

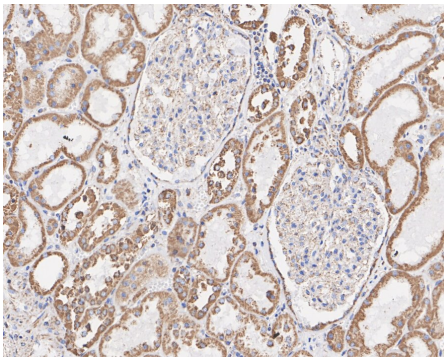

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/1,000 dilution.

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 (ET1601-20) 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.

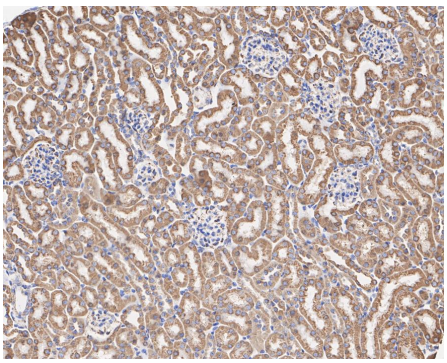

Fig7: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/1,000 dilution.

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 (ET1601-20) 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.

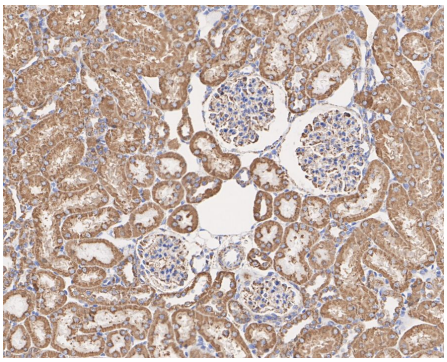

Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-VDAC1 antibody (ET1601-20) at 1/1,000 dilution.

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 (ET1601-20) 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.

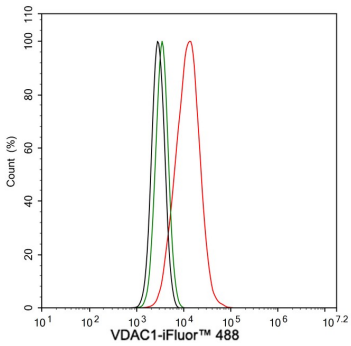

Fig9: Flow cytometric analysis of HeLa cells labeling VDAC1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-20, 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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. "Influenza virus PB1-F2 protein induces cell death through mitochondrial ANT3 and VDAC1."Zamarin D., Garcia-Sastre A., Xiao X., Wang R., Palese P. PLoS Pathog. 1:40-54(2005).
2. "Solution structure of the integral human membrane protein VDAC-1 in detergent micelles." Hiller S., Garces R.G., Malia T.J., Orekhov V.Y., Colombini M., Wagner G. Science 321:1206-1210(2008).