Anti-TOMM20 Antibody [ST04-72]

ET1609-25

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IF-Cell, IP, IHC-Fr, FC(Intra)
Molecular Wt:	Predicted band size: 16 kDa
Clone number:	ST04-72

Description: Mitochondrial import receptor subunit TOM20 homolog is a protein that in humans is encoded by the TOMM20 gene. TOM20 is one of the receptor systems of the translocase of the outer membrane (TOM) complex in the outer mitochondrial membrane. In mitochondrial protein import, TOM20 is closely associated with the pore-forming TOM40 complex and acts by recognizing and binding the N-terminal MTSs (matrix-targeting sequences), which form an amphipathic alpha helix and aid passage of the target proteins into the mitochondrial matrix.

Immunogen: Recombinant protein within Human TOMM20 aa 1-145 / 145.

Positive control: HeLa cell lysate, Saos-2 cell lysate, HepG2 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, MCF7 cell lysate, F9 cell lysate, Rat lung tissue lysate, HepG2, NIH/3T3, HeLa, human kidney tissue, rat kidney tissue, mouse kidney tissue, human liver tissue, rat large intestine tissue.

Subcellular location: Mitochondrion outer membrane.

Database links: SwissProt: Q15388 Human | Q9DCC8 Mouse | Q62760 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IHC-P	1:500-1:1,000
IHC-Fr	1:500
IF-Tissue	1:500
IF-Cell	1:1,000
IP	Use at an assay dependent concentration.
FC(Intra)	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

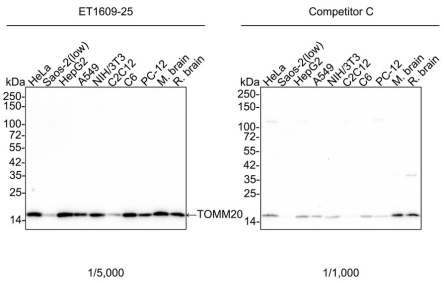

Fig1: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Saos-2 cell lysate (low expression) (15 µg/Lane)
Lane 3: HepG2 cell lysate (15 µg/Lane)
Lane 4: A549 cell lysate (15 µg/Lane)
Lane 5: NIH/3T3 cell lysate (15 µg/Lane)
Lane 6: C2C12 cell lysate (15 µg/Lane)
Lane 7: C6 cell lysate (15 µg/Lane)
Lane 8: PC-12 cell lysate (15 µg/Lane)
Lane 9: Mouse brain tissue lysate (30 µg/Lane)
Lane 10: Rat brain tissue lysate (30 µg/Lane)

Predicted band size: 16 kDa
Observed band size: 16 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 (ET1609-25) at 1/5,000 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.

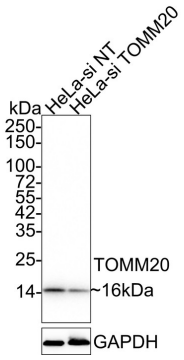

Fig2: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/5,000 dilution.

Lane 1: HeLa-si NT cell lysate (10 µg/Lane)
Lane 2: HeLa-si TOMM20 cell lysate (10 µg/Lane)

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: 21 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 (ET1609-25) at 1/5,000 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.

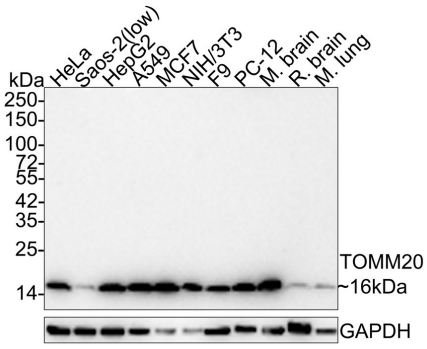

Fig3: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: Saos-2 cell lysate (low expression) (10 µg/Lane)
Lane 3: HepG2 cell lysate (10 µg/Lane)
Lane 4: A549 cell lysate (10 µg/Lane)
Lane 5: MCF7 cell lysate (10 µg/Lane)
Lane 6: NIH/3T3 cell lysate (10 µg/Lane)
Lane 7: F9 cell lysate (10 µg/Lane)
Lane 8: PC-12 cell lysate (10 µg/Lane)
Lane 9: Mouse brain tissue lysate (20 µg/Lane)
Lane 10: Rat brain tissue lysate (20 µg/Lane)
Lane 11: Rat lung tissue lysate (20 µg/Lane)

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: 1 minute 22 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 (ET1609-25) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

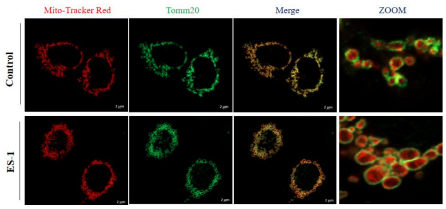

Fig4: Application: IF-cell

Species: Human

Cell Sample: HeLa cell

Antibody concentration: 1: 1,000

Data by conrtesy of: Mr. Wenxiang Huang
School of Basic Medical Sicences, Zhejiang University

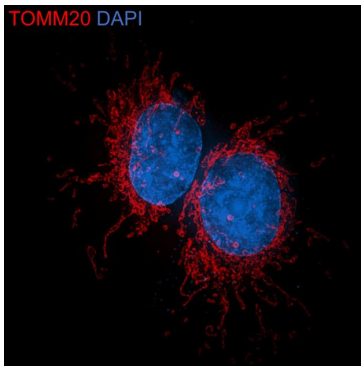

Fig5: Application: IF-cell

Species: Human

Cell Sample: AC16 cell

Antibody concentration: 1: 200

Data by conrtesy of: Mr. Zhiyi Yang
School of Basic Medical Sicences, Zhejiang University

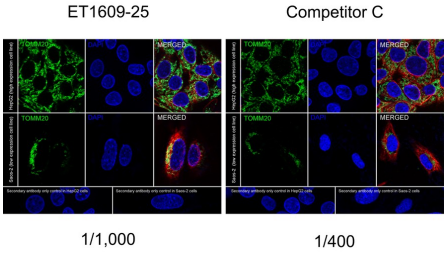

Fig6: Immunocytochemistry analysis of HepG2 (high expression) and Saos-2 (low expression) labeling TOMM20 with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 dilution.

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-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 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.

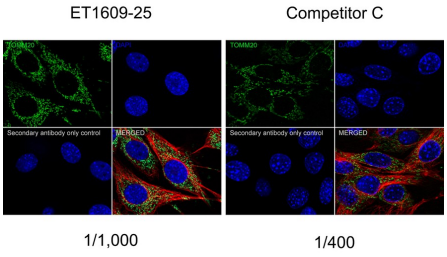

Fig7: Immunocytochemistry analysis of NIH/3T3 cells labeling TOMM20 with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 dilution.

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-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 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.

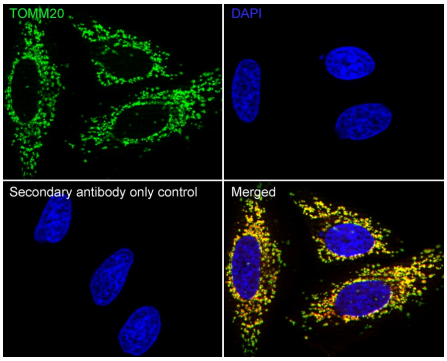

Fig8: Immunocytochemistry analysis of HeLa cells labeling TOMM20 with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-TOMM20 antibody (ET1609-25) at 1/1,000 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

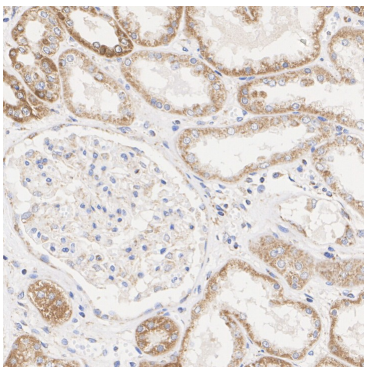

Fig9: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/500 dilution.

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 (ET1609-25) at 1/500 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.

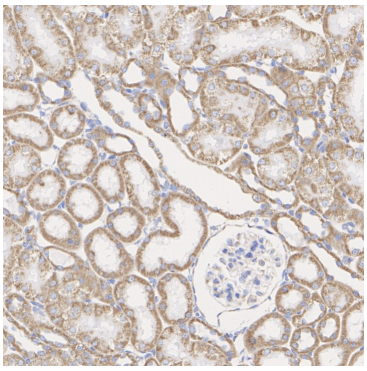

Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/500 dilution.

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 (ET1609-25) at 1/500 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.

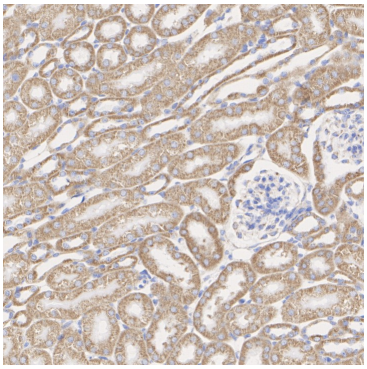

Fig11: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/500 dilution.

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 (ET1609-25) at 1/500 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.

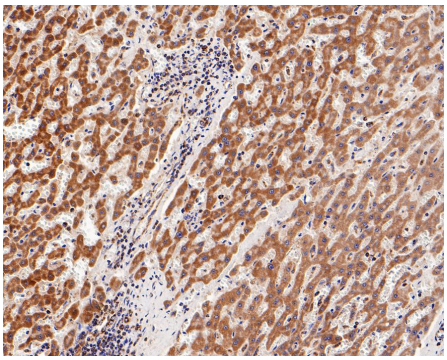

Fig12: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/800 dilution.

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 (ET1609-25) at 1/800 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.

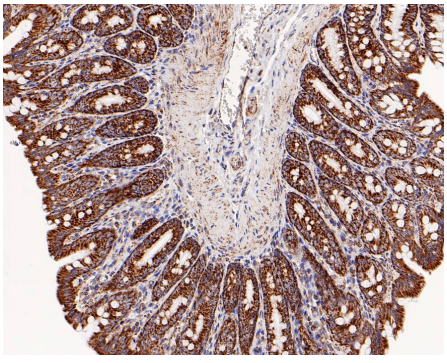

Fig13: Immunohistochemical analysis of paraffin-embedded rat large intestine tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/800 dilution.

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 (ET1609-25) at 1/800 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.

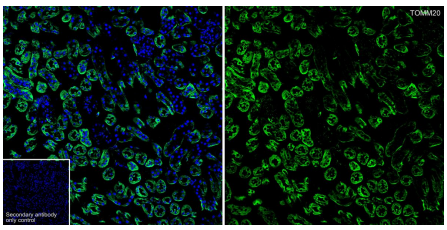

Fig14: Application: IHC-Fr

Species: Mouse

Site: Kidney

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

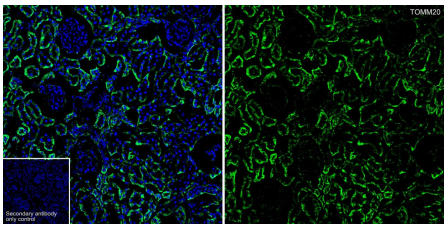

Fig15: Application: IHC-Fr

Species: Rat

Site: Kidney

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

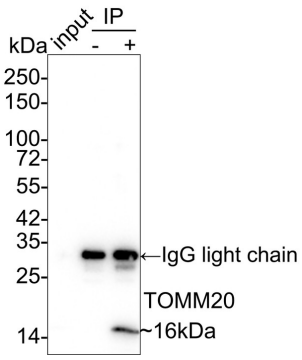

Fig16: TOMM20 was immunoprecipitated in 0.2mg HeLa cell lysate with ET1609-25 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1609-25 at 1/1,000 dilution. Anti-Rabbit IgG for IP, AlpSdAbs® VHH(HRP) (025-100-005) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: Rabbit IgG instead of ET1609-25 in HeLa cell lysate
Lane 3: ET1609-25 IP in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 2 seconds

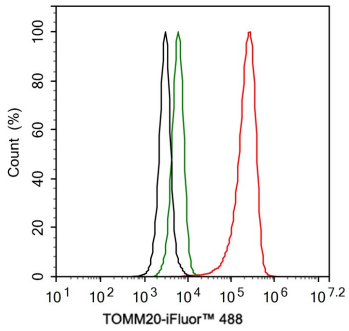

Fig17: Flow cytometric analysis of HeLa cells labeling TOMM20.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1609-25, 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).

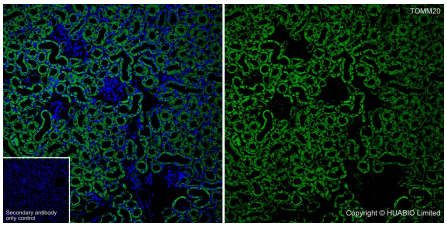

Fig18: Application: Immunofluorescence (IF-tissue)

Species: Mouse
Tissue: kidney
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95℃.

Wash buffer: 1× PBS
Endogenous peroxidase blocking: 3% H O , 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: ET1609-25, 1/500, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

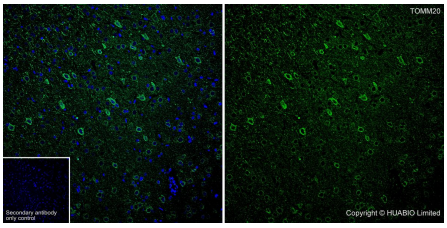

Fig19: Application: Immunofluorescence (IF-tissue)

Species: Rat
Tissue: brain
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95℃.

Wash buffer: 1× PBS
Endogenous peroxidase blocking: 3% H O , 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: ET1609-25, 1/500, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

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

Background References

1. Micaily I et al. TOMM20 as a Potential Prognostic Biomarker in Chordoma: Results From a High-Volume, Single-Center Study. Am J Clin Pathol. 2023 May
2. Yin L et al. AR antagonists develop drug resistance through TOMM20 autophagic degradation-promoted transformation to neuroendocrine prostate cancer. J Exp Clin Cancer Res. 2023 Aug