Anti-TOMM20 Antibody [ST04-72]

ET1609-25

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

Description: The mitochondrial preprotein translocases of the outer membrane (Tom) is a multisubunit protein complex that facilitates the import of nucleus-encoded precursor proteins across the mitochondrial outer membrane. The Tom machinery consists of import receptors for the initial binding of cytosolically synthesized preproteins and a general import pore (GIP) for the membrane translocation of various preproteins into the mitochondria. The import receptors include Tom20 and Tom22, which form a heteromeric receptor complex that initiates the insertion of newly synthesized proteins into the outer membrane and then directs the precursor protein into the GIP. In yeast, Tom22 is the essential component of the import receptor complex as it functions as both a receptor for the preproteins and serves as a docking point for both Tom20 and the GIP. Tom22 directly associates with Tom40, the major component of the GIP, and thereby forms a stable interaction between the two core complexes to facilitate the fluid movement of preproteins into the mitochondria. The insertion of Tom40 into the Tom machinery requires the initial binding of Tom40 to Tom20 and leads to the efficient incorporation of Tom40 precursors into preexisting Tom complexes.

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, HepG2, MCF7 cell lysate, F9 cell lysate, rat lung tissue lysate, NIH/3T3, human liver carcinoma tissue, human kidney tissue, mouse kidney tissue, mouse small intestine tissue, mouse heart tissue, rat large intestine tissue, human liver tissue, HeLa.

Subcellular location: Mitochondrion outer membrane.

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

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:1,000
IF-Tissue	1:50-1:200
IHC-P	1:50-1:800
FC	1:50-1:100
IP	Use at an assay dependent concentration.

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

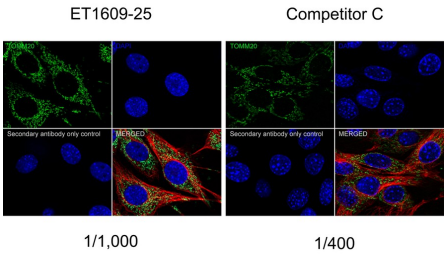

Fig1: Immunocytochemistry analysis of NIH/3T3 cells labeling TOMM20 with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 dilution.

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.

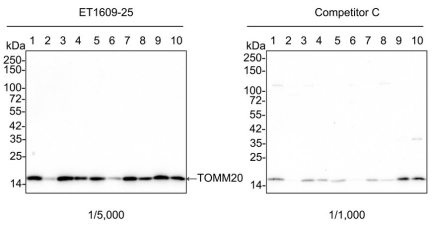

Fig2: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Saos-2 cell lysate (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;

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.

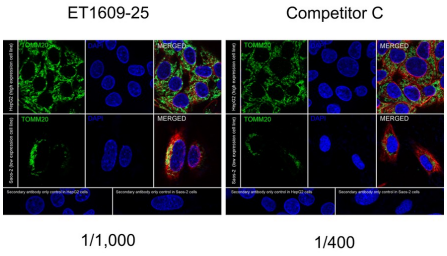

Fig3: Immunocytochemistry analysis of HepG2 (high) and Saos-2 (low) labeling TOMM20 with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/1,000 dilution and competitor's antibody at 1/400 dilution.

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.

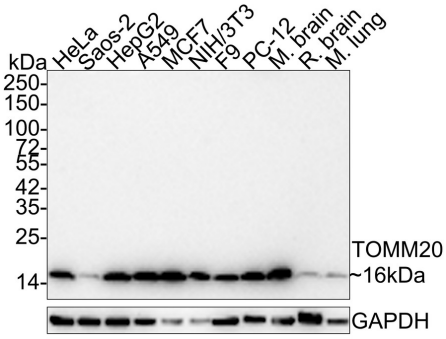

Fig4: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: Saos-2 cell lysate (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;

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 1/2,000 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.

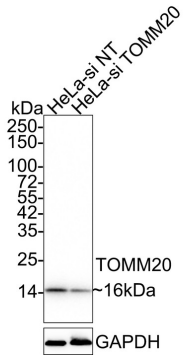

Fig5: Western blot analysis of TOMM20 on different lysates with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/5,000 dilution.

Lane 1: HeLa-si NT cell lysate
Lane 2: HeLa-si TOMM20 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 21 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 (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.

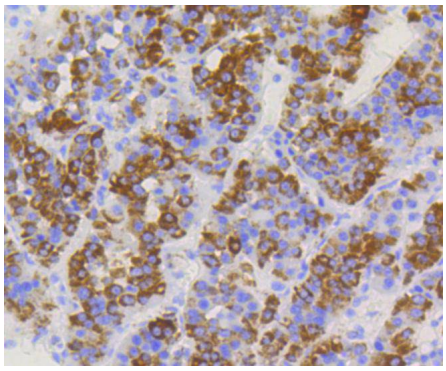

Fig6: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-TOMM20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-25, 1/50) for 30 minutes 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.

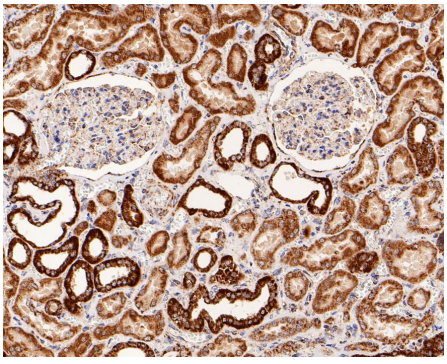

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/800 dilution.

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.

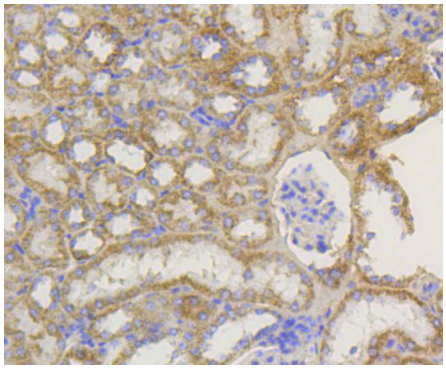

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-TOMM20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-25, 1/50) for 30 minutes 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.

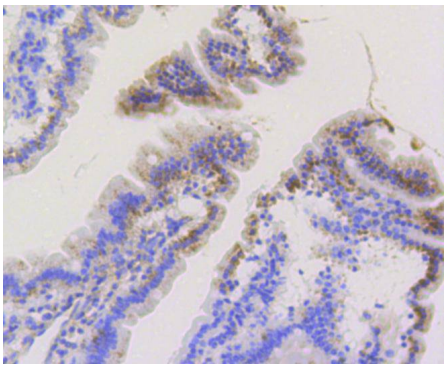

Fig9: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue using anti-TOMM20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-25, 1/50) for 30 minutes 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.

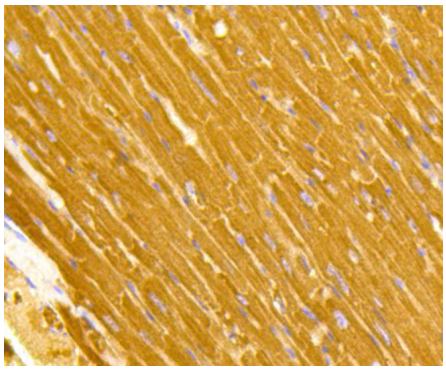

Fig10: Immunohistochemical analysis of paraffin-embedded mouse heart tissue using anti-TOMM20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-25, 1/50) for 30 minutes 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.

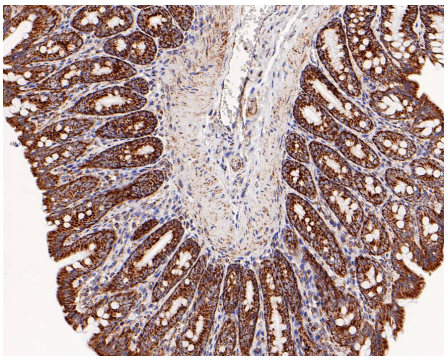

Fig11: Immunohistochemical analysis of paraffin-embedded rat large intestine tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/800 dilution.

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.

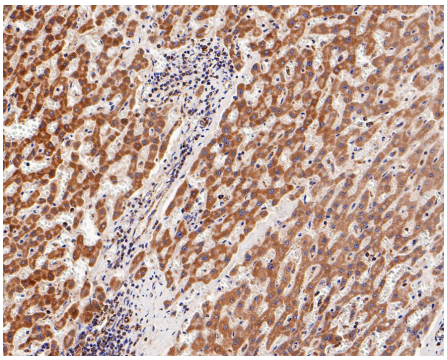

Fig12: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-TOMM20 antibody (ET1609-25) at 1/800 dilution.

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.

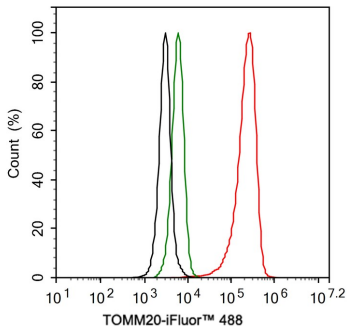

Fig13: Flow cytometric analysis of HeLa cells labeling TOMM20.

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).

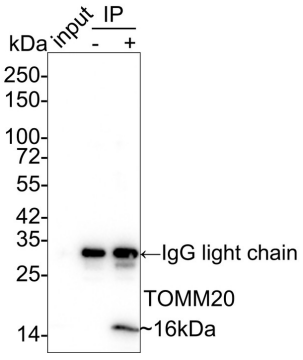

Fig14: TOMM20 was immunoprecipitated in 0.2mg HeLa cell lysate with ET1609-25 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1609-25 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

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

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

Background References

1. Kim SJ et al. Hepatitis C virus induces the mitochondrial translocation of Parkin and subsequent mitophagy. PLoS Pathog 9:e1003285 (2013).
2. Kim SJ et al. Hepatitis B virus disrupts mitochondrial dynamics: induces fission and mitophagy to attenuate apoptosis. PLoS Pathog 9:e1003722 (2013).