Anti-mTOR Antibody [SU30-00]

ET1608-5

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IP, IF-Cell
Molecular Wt:	Predicted band size: 289 kDa
Clone number:	SU30-00

Description: The mammalian target of rapamycin (mTOR), also referred to as the mechanistic target of rapamycin, and sometimes called FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1), is a kinase that in humans is encoded by the MTOR gene. mTOR is a member of the phosphatidylinositol 3-kinase-related kinase family of protein kinases. mTOR links with other proteins and serves as a core component of two distinct protein complexes, mTOR complex 1 and mTOR complex 2, which regulate different cellular processes. In particular, as a core component of both complexes, mTOR functions as a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription. As a core component of mTORC2, mTOR also functions as a tyrosine protein kinase that promotes the activation of insulin receptors and insulin-like growth factor 1 receptors. mTORC2 has also been implicated in the control and maintenance of the actin cytoskeleton.

Immunogen: Synthetic peptide within human mTOR aa 2400-2440.

Positive control: HeLa cell lysate, MCF7 cell lysate, HEK-293 cell lysate, HepG2 cell lysate, C2C12 cell lysate, PC-12 cell lysate, C6 cell lysate, mouse testis tissue lysate, rat testis tissue lysate, rat heart tissue lysate, SiHa cell lysate, human breast carcinoma tissue, HeLa, NIH/3T3, human kidney tissue, mouse testis tissue, mouse kidney tissue.

Subcellular location: Endoplasmic reticulum membrane, Microsome membrane, PML body, Golgi apparatus membrane, Cytoplasm, Lysosome, Lysosome membrane, Mitochondrion outer membrane, phagosome.

Database links: SwissProt: P42345 Human | Q9JLN9 Mouse | P42346 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:200-1:1,000
IF-cell	1:50-1:200
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

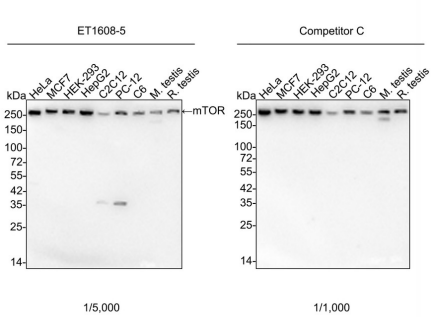

Fig1: Western blot analysis of mTOR on different lysates with Rabbit anti-mTOR antibody (ET1608-5) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: MCF7 cell lysate (20 µg/Lane)
Lane 3: HEK-293 cell lysate (20 µg/Lane)
Lane 4: HepG2 cell lysate (20 µg/Lane)
Lane 5: C2C12 cell lysate (20 µg/Lane)
Lane 6: PC-12 cell lysate (20 µg/Lane)
Lane 7: C6 cell lysate (20 µg/Lane)
Lane 8: Mouse testis tissue lysate (20 µg/Lane)
Lane 9: Rat testis tissue lysate (20 µg/Lane)

Predicted band size: 289 kDa
Observed band size: 289 kDa

Exposure time: 24 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 (ET1608-5) 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.

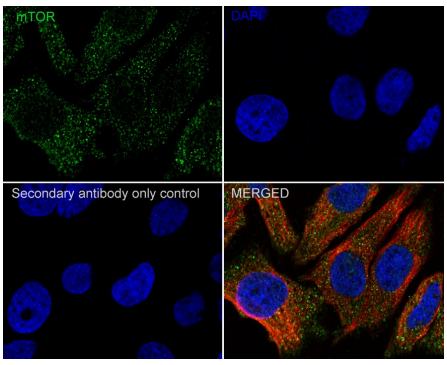

Fig2: Immunocytochemistry analysis of HeLa cells labeling mTOR with Rabbit anti-mTOR antibody (ET1608-5) at 1/100 dilution.

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-mTOR antibody (ET1608-5) 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.

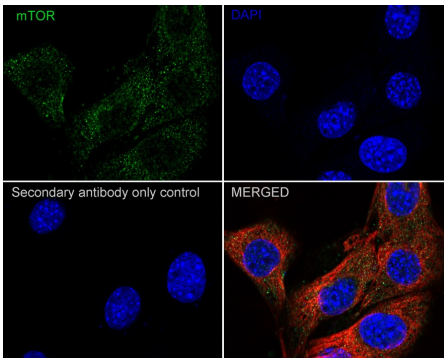

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling mTOR with Rabbit anti-mTOR antibody (ET1608-5) at 1/200 dilution.

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-mTOR antibody (ET1608-5) at 1/200 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.

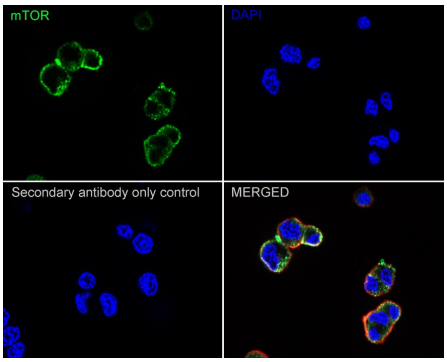

Fig4: Immunocytochemistry analysis of PC-12 cells labeling mTOR with Rabbit anti-mTOR antibody (ET1608-5) at 1/100 dilution.

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-mTOR antibody (ET1608-5) 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.

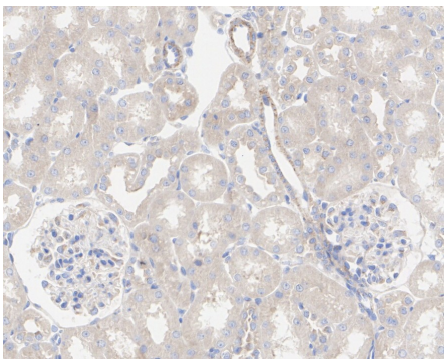

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-mTOR antibody (ET1608-5) at 1/5,000 dilution.

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 (ET1608-5) 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.

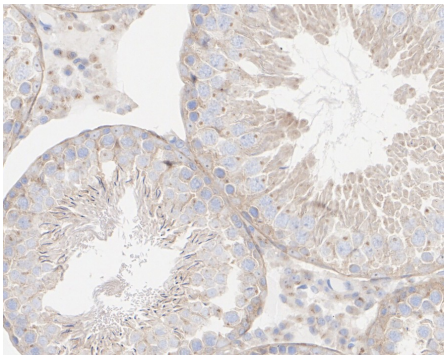

Fig6: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-mTOR antibody (ET1608-5) at 1/5,000 dilution.

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 (ET1608-5) 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.

Background References

1. Atif F et al. Anti-tumor effects of progesterone in human glioblastoma multiforme: role of PI3K/Akt/mTOR signaling. J Steroid Biochem Mol Biol 146:62-73 (2015).
2. Atif F et al. The Synergistic Effect of Combination Progesterone and Temozolomide on Human Glioblastoma Cells. PLoS One 10:e0131441 (2015).