

# Anti-MLST8 Antibody

## HA500372



<b>Product Type:</b>	Rabbit polyclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 36 kDa

**Description:** Subunit of both mTORC1 and mTORC2, which regulates cell growth and survival in response to nutrient and hormonal signals. mTORC1 is activated in response to growth factors or amino acids. Growth factor-stimulated mTORC1 activation involves a AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of mTORC1. Amino acid-signaling to mTORC1 requires its relocalization to the lysosomes mediated by the Ragulator complex and the Rag GTPases. Activated mTORC1 up-regulates protein synthesis by phosphorylating key regulators of mRNA translation and ribosome synthesis. mTORC1 phosphorylates EIF4EBP1 and releases it from inhibiting the elongation initiation factor 4E (eIF4E). mTORC1 phosphorylates and activates S6K1 at 'Thr-389', which then promotes protein synthesis by phosphorylating PDCD4 and targeting it for degradation. Within mTORC1, LST8 interacts directly with MTOR and enhances its kinase activity. In nutrient-poor conditions, stabilizes the MTOR-RPTOR interaction and favors RPTOR-mediated inhibition of MTOR activity. mTORC2 is also activated by growth factors, but seems to be nutrient-insensitive. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress-fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PRKCA on 'Ser-657'.

**Immunogen:** Recombinant protein within human MLST8 aa 1-250.

**Positive control:** C2C12 cell lysate, L6 cell lysate, mouse brain tissue lysate, human brain tissue lysate, rat brain tissue lysate, 293 cell lysate, MCF-7 cell lysate, PC-3M, rat heart tissue, mouse skeletal muscle tissue, PC-3.

**Subcellular location:** Cytoplasm.

**Database links:** SwissProt: Q9BVC4 Human | Q9DCJ1 Mouse | Q9Z2K5 Rat

**Recommended Dilutions:**

<b>WB</b>	1:500-1:1,000
<b>IF-Cell</b>	1:200
<b>IHC-P</b>	1:800
<b>FC</b>	1:1,000

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

**Purity:** Immunogen affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

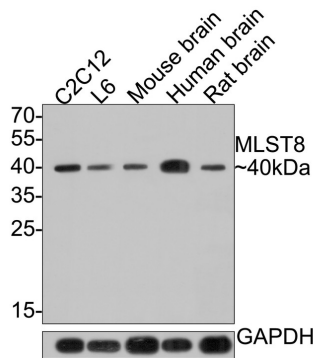
Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物  
HUABIO  
www.huabio.cn

## Images



**Fig1:** Western blot analysis of MLST8 on different lysates with Rabbit anti-MLST8 antibody (HA500372) at 1/1,000 dilution.

Lane 1: C2C12 cell lysate (10 µg/Lane)  
 Lane 2: L6 cell lysate (10 µg/Lane)  
 Lane 3: Mouse brain tissue lysate (20 µg/Lane)  
 Lane 4: Human brain tissue lysate (20 µg/Lane)  
 Lane 5: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 40 kDa  
 Observed band size: 36 kDa

Exposure time: 2 minutes;

12% 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 (HA500372) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

**Fig2:** Western blot analysis of MLST8 on different lysates with Rabbit anti-MLST8 antibody (HA500372) at 1/500 dilution.

Lane 1: 293 cell lysate  
 Lane 2: MCF-7 cell lysate

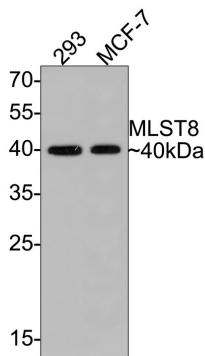
Lysates/proteins at 10 µg/Lane.

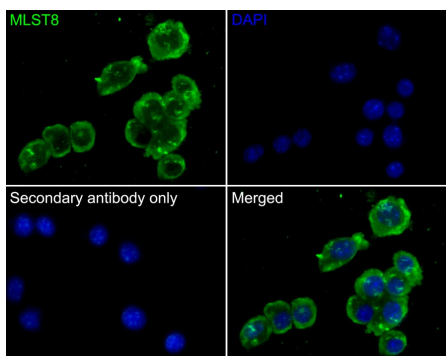
Predicted band size: 40 kDa  
 Observed band size: 36 kDa

Exposure time: 2 minutes;

12% SDS-PAGE gel.

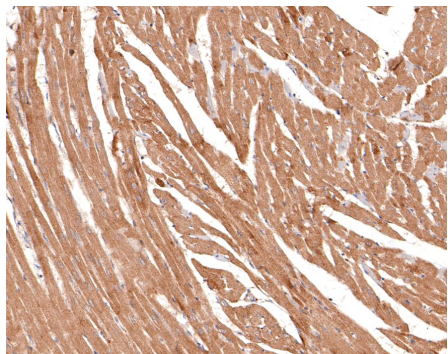
Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA500372) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.





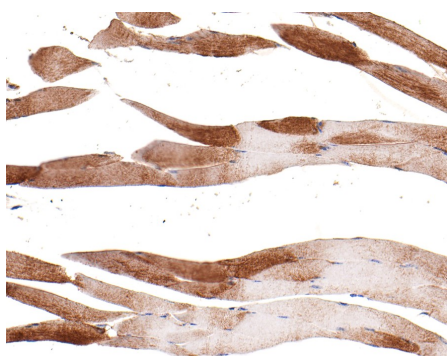
**Fig3:** Immunocytochemistry analysis of PC-3M cells labeling MLST8 with Rabbit anti-MLST8 antibody (HA500372) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, 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-MLST8 antibody (HA500372) at 1/200 dilution in 2% negative goat serum overnight at 4 °C. 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.



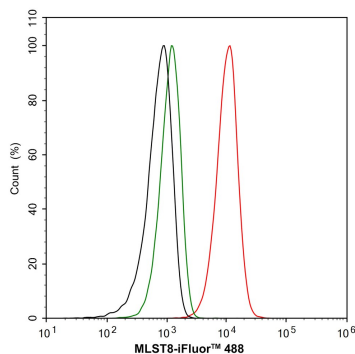
**Fig4:** Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-MLST8 antibody (HA500372) at 1/800 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500372) 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.



**Fig5:** Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue with Rabbit anti-MLST8 antibody (HA500372) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500372) 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.



**Fig6:** Flow cytometric analysis of PC-3 cells labeling MLST8.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500372, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. 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. Kim D.-H., Sarbassov D.D., Ali S.M., Latek R.R., Guntur K.V.P., Erdjument-Bromage H., Tempst P., Sabatini D.M. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol. Cell* 11:895-904(2003).
2. Jacinto E., Loewith R., Schmidt A., Lin S., Ruegg M.A., Hall A., Hall M.N. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* 6:1122-1128(2004).

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn


 华安生物  
 HUABIO  
[www.huabio.cn](http://www.huabio.cn)