Anti-M6PR Antibody [SR45-09]

ET1602-5

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: 274 kDa
Clone number:	SR45-09

Description: The mannose 6-phosphate/insulin-like growth factor II receptor, IGF-IIR (also designated M6P/IGF2R), is a ubiquitously expressed integral glycoprotein. By binding glycoproteins through two of its extracytoplasmic domains, IGF-IIR mediates the activation of TGF∫1 (a growth inhibitor), the degradation of IGF-II and the transport of lysosomal enzymes. Subsequently, IGF-IIR can form oligomeric complexes, which increase the affinity of IGF-IIR for lysosomal enzymes. Unlike IGF-IR, IGF-IIR does not potentiate the signaling of IGF-I or IGF-II, which have mitogenic, cell survival and insulin-like effects. Therefore, IGF-IIR is characterized as a tumor suppressor. Furthermore, the IGF-IIR gene is located on chromosome 6q26, which is commonly mutated or deleted in several human cancers.

Immunogen: Synthetic peptide within Human M6PR aa 2,442-2,491 / 2,491.

Positive control: HeLa cell lysate, SK-OV-3 cell lysate, SiHa cell lysate, SK-MEL-28 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, PC-12 cell lysate, HeLa, NIH/3T3, C6, human liver tissue, human tonsil tissue, mouse colon tissue, rat colon tissue.

Subcellular location: Golgi apparatus membrane, Endosome membrane.

Database links: SwissProt: P11717 Human | Q07113 Mouse
Entrez Gene: 25151 Rat

Recommended Dilutions:

WB	1:2,000-1:10,000
IF-Cell	1:100-1:250
IF-Tissue	1:50-1:200
IHC-P	1:200
FC	1:1,000
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: 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

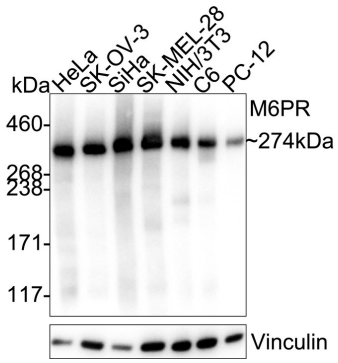

Fig1: Western blot analysis of M6PR on different lysates with Rabbit anti-M6PR antibody (ET1602-5) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: SK-OV-3 cell lysate
Lane 3: SiHa cell lysate
Lane 4: SK-MEL-28 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C6 cell lysate
Lane 7: PC-12 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 274 kDa
Observed band size: 274 kDa

Exposure time: 6 seconds; ECL: K1801;

3-8% 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 (ET1602-5) 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.

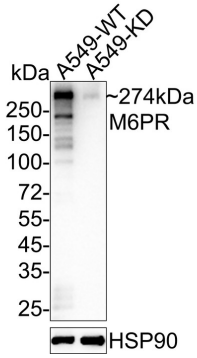

Fig2: Western blot analysis of M6PR on different lysates with Rabbit anti-M6PR antibody (ET1602-5) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si M6PR cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 274 kDa
Observed band size: 274 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 (ET1602-5) at 1/2,000 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.

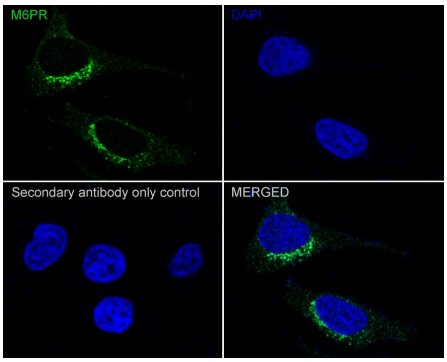

Fig3: Immunocytochemistry analysis of HeLa cells labeling M6PR with Rabbit anti-M6PR antibody (ET1602-5) at 1/100 dilution.

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-M6PR antibody (ET1602-5) 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.

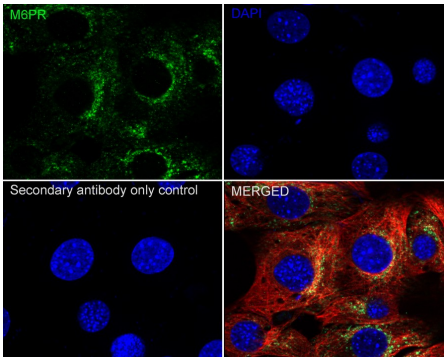

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling M6PR with Rabbit anti-M6PR antibody (ET1602-5) at 1/100 dilution.

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-M6PR antibody (ET1602-5) 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.

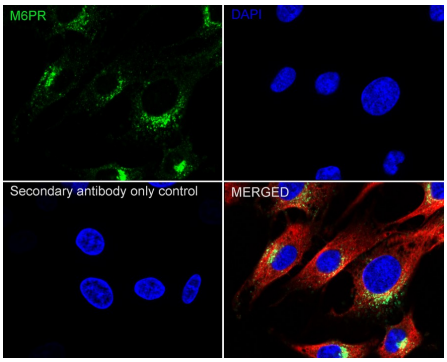

Fig5: Immunocytochemistry analysis of C6 cells labeling M6PR with Rabbit anti-M6PR antibody (ET1602-5) at 1/250 dilution.

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-M6PR antibody (ET1602-5) at 1/250 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.

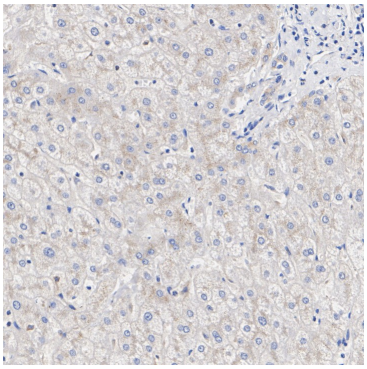

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-M6PR antibody (ET1602-5) at 1/200 dilution.

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 (ET1602-5) at 1/200 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.

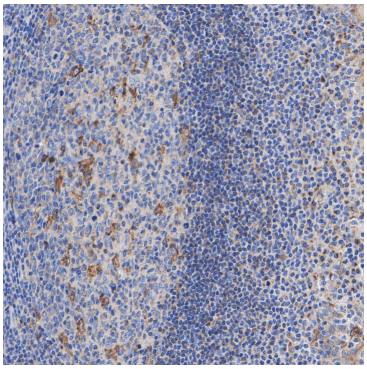

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-M6PR antibody (ET1602-5) at 1/200 dilution.

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 (ET1602-5) at 1/200 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.

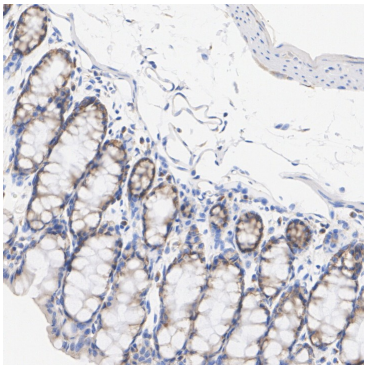

Fig8: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-M6PR antibody (ET1602-5) at 1/200 dilution.

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 (ET1602-5) at 1/200 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.

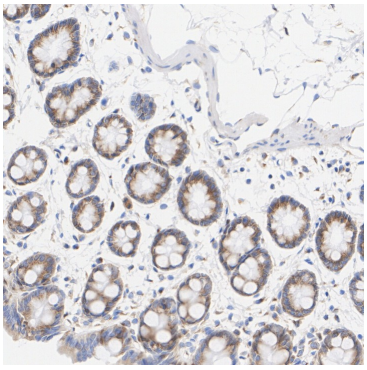

Fig9: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-M6PR antibody (ET1602-5) at 1/200 dilution.

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 (ET1602-5) at 1/200 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.

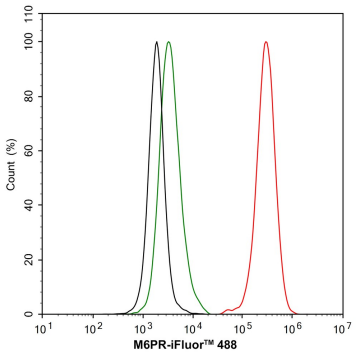

Fig10: Flow cytometric analysis of HeLa cells labeling M6PR.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-5, 1/1,000) (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. Tsika E et al. Parkinson's disease-linked mutations in VPS35 induce dopaminergic neurodegeneration. Hum Mol Genet N/A:N/A (2014).
2. Alsalem JA et al. Characterization of vitamin D production by human ocular barrier cells. Invest Ophthalmol Vis Sci 55:2140-7 (2014).