

# Anti-PSMB5 / MB1 Antibody

## HA500533



<b>Product Type:</b>	Rabbit polyclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat, Monkey
<b>Applications:</b>	WB, IHC-P, IF-Cell, FC
<b>Molecular Wt:</b>	Predicted band size: 28 kDa

**Description:** The proteasome is a multicatalytic proteinase complex with a highly ordered ring-shaped 20S core structure. The core structure is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes a member of the proteasome B-type family, also known as the T1B family, that is a 20S core beta subunit in the proteasome. This catalytic subunit is not present in the immunoproteasome and is replaced by catalytic subunit 3i (proteasome beta 8 subunit). Multiple transcript variants encoding different isoforms have been found for this gene.

**Immunogen:** Recombinant protein within human PSMB5 aa 34-263 / 263.

**Positive control:** HeLa cell lysate, MCF7 cell lysate, K-562 cell lysate, HEK-293 cell lysate, COS-1 cell lysate, Neuro-2a cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Mouse kidney tissue lysate, Rat kidney tissue lysate, A431, HeLa, human kidney tissue, human liver tissue, mouse kidney tissue, rat kidney tissue, human breast carcinoma tissue, mouse liver tissue, rat brain tissue.

**Subcellular location:** Cytoplasm, Nucleus.

**Database links:** SwissProt: P28074 Human | O55234 Mouse | P28075 Rat

**Recommended Dilutions:**

<b>WB</b>	1:10,000
<b>IHC-P</b>	1:2,000
<b>IF-Cell</b>	1:200
<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.

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Orders:0086-571-88062880

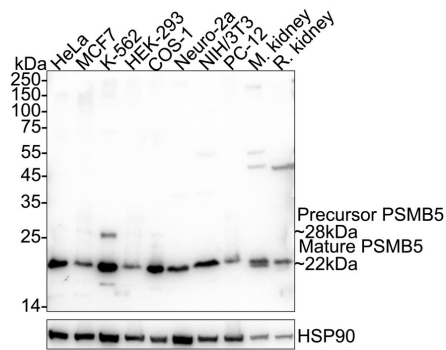
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## Images

**Fig1:** Western blot analysis of PSMB5 / MB1 on different lysates with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/10,000 dilution.



Lane 1: HeLa cell lysate (20 µg/Lane)  
 Lane 2: MCF7 cell lysate (20 µg/Lane)  
 Lane 3: K-562 cell lysate (20 µg/Lane)  
 Lane 4: HEK-293 cell lysate (20 µg/Lane)  
 Lane 5: COS-1 cell lysate (20 µg/Lane)  
 Lane 6: Neuro-2a cell lysate (20 µg/Lane)  
 Lane 7: NIH/3T3 cell lysate (20 µg/Lane)  
 Lane 8: PC-12 cell lysate (20 µg/Lane)  
 Lane 9: Mouse kidney tissue lysate (40 µg/Lane)  
 Lane 10: Rat kidney tissue lysate (40 µg/Lane)

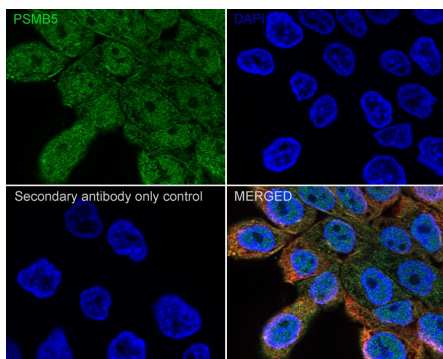
Predicted band size: 28 kDa  
 Observed band size: 28/22 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA500533) at 1/10,000 dilution was used in primary antibody dilution (K1803) at 4°C 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 A431 cells labeling PSMB5 / MB1 with Rabbit anti-PSMB5 / MB1 antibody (HA500533) 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-PSMB5 / MB1 antibody (HA500533) at 1/200 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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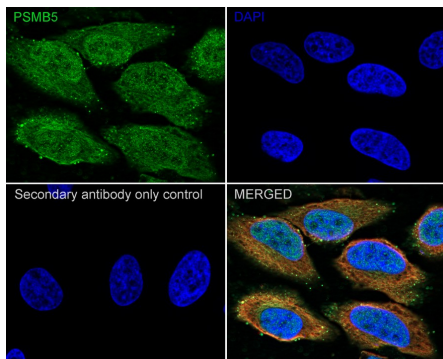
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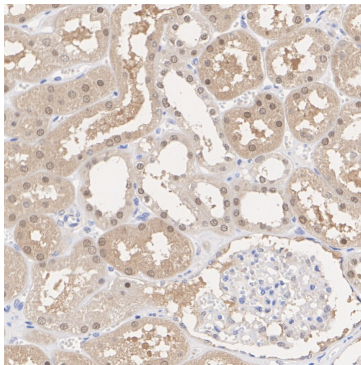
**Fig3:** Immunocytochemistry analysis of HeLa cells labeling PSMB5 / MB1 with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/200 dilution.



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-PSMB5 / MB1 antibody (HA500533) at 1/200 dilution in 1% BSA in PBST 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.

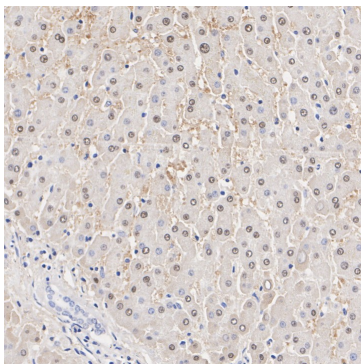
Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig4:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

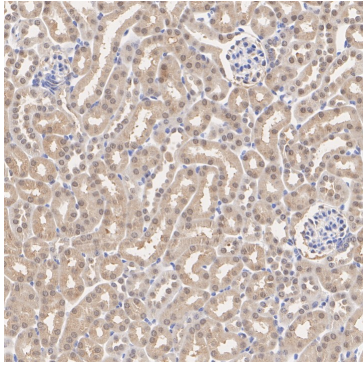


The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500533) at 1/2,000 dilution for 1 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.

**Fig5:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

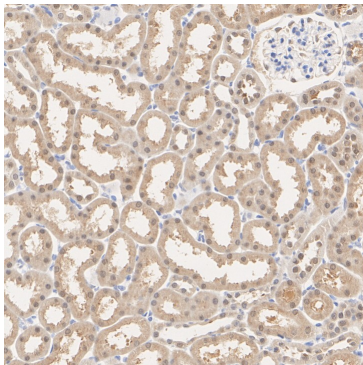


The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500533) at 1/2,000 dilution for 1 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.



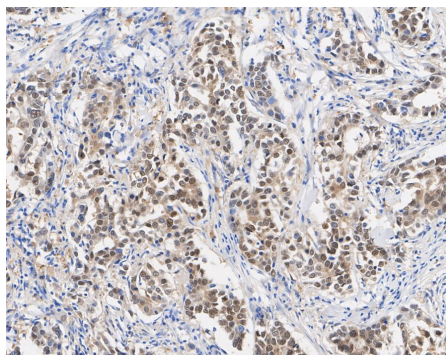
**Fig6:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500533) at 1/2,000 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.



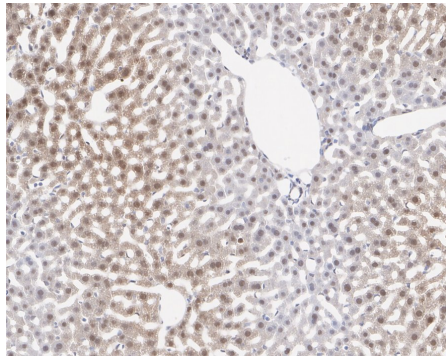
**Fig7:** Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500533) at 1/2,000 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.



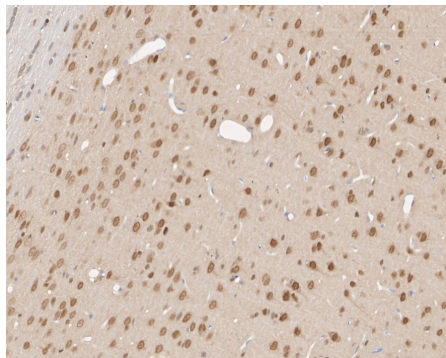
**Fig8:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA500533) at 1/2,000 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.



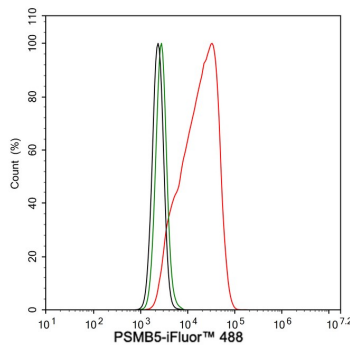
**Fig9:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA500533) at 1/2,000 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.



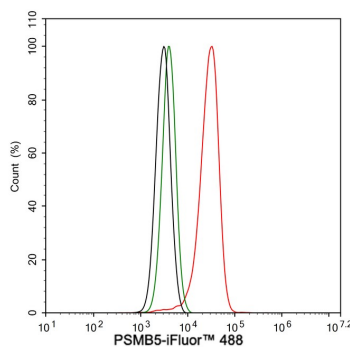
**Fig10:** Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA500533) at 1/2,000 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.



**Fig11:** Flow cytometric analysis of A431 cells labeling PSMB5 / MB1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500533, 1 $\mu$ g/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor<sup>™</sup> 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig12:** Flow cytometric analysis of HeLa cells labeling PSMB5 / MB1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500533, 1 $\mu$ g/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor<sup>™</sup> 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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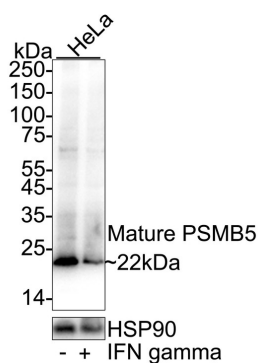
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**Fig13:** Western blot analysis of PSMB5 / MB1 on different lysates with Rabbit anti-PSMB5 / MB1 antibody (HA500533) at 1/10,000 dilution.

Lane 1: HeLa cell lysate

Lane 2: HeLa treated with 100ng/mL IFN gamma for 72 hours cell lysate



Lysates/proteins at 15 µg/Lane.

Predicted band size: 28 kDa

Observed band size: 22 kDa

Exposure time: 10 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 (HA500533) at 1/10,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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

### Background References

1. Han L et al. PSMB5 Alleviates Ulcerative Colitis by Inhibiting ROS-Dependent NLRP3 Inflammasome-Mediated Pyroptosis. *Dis Markers*. 2022 Aug
2. Liu J et al. PSMB5 overexpression is correlated with tumor proliferation and poor prognosis in hepatocellular carcinoma. *FEBS Open Bio*. 2022 Nov

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