abcam

Product datasheet

Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] ab180606

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 7 References 11 Images

Overview

Product name	Anti-Proteasome 20S LMP7 antibody [EPR14482(B)]
Description	Rabbit monoclonal [EPR14482(B)] to Proteasome 20S LMP7
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A549, Raji, Jurkat, HeLa and U937 lysates. IHC-P: Human bladder transitional cell carcinoma tissue. ICC/IF: Jurkat and HeLa cells. Flow Cyt (intra): Jurkat cells. IP: Proteasome 20S LMP7 IP in Raji cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR14482(B)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab180606 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/1700. **For unpurified use at 1/250 - 1/500.** Perform heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0)
WB		1/10000 - 1/50000. Detects a band of approximately 23 kDa (predicted molecular weight: 30 kDa).
ICC/IF		1/100.
Flow Cyt (Intra)		1/90 - 1/150. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for IP.

Target

Function The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. This subunit is involved in antigen processing to generate class I binding peptides. Replacement of PSMB5 by PSMB8 increases the capacity of the immunoproteasome to cleave model peptides after hydrophobic and basic residues. Acts as a major component of interferon gamma-induced sensitivity. Plays a key role in apoptosis via the degradation of the apoptotic inhibitor MCL1. May be involved in the inflammatory response pathway. In cancer cells, substitution of isoform 1 (E2) by isoform 2 (E1) results in immunoproteasome deficiency.

Involvement in disease Defects in PSMB8 are the cause of JMP syndrome (JMPS) [MIM:613732]; also called joint contractures muscular atrophy microcytic anemia and panniculitis-induced lipodystrophy. JBTS1 is an autoinflammatory disorder characterized by childhood onset of joint stiffness and severe contractures of the hands and feet, erythematous skin lesions with subsequent development of severe lipodystrophy, and laboratory evidence of immune dysregulation. Accompanying features include muscle weakness and atrophy, hepatosplenomegaly, and microcytic anemia.

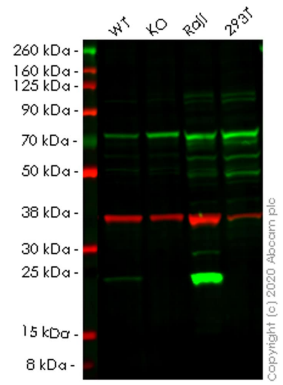
Sequence similarities Belongs to the peptidase T1B family.

Developmental stage Highly expressed in immature dendritic cells (at protein level).

Post-translational modifications Autocleaved. The resulting N-terminal Thr residue of the mature subunit is responsible for the nucleophile proteolytic activity.

Cellular localization Cytoplasm. Nucleus.

Images



Western blot - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

All lanes : Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : PSMB8 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate
Lane 4 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

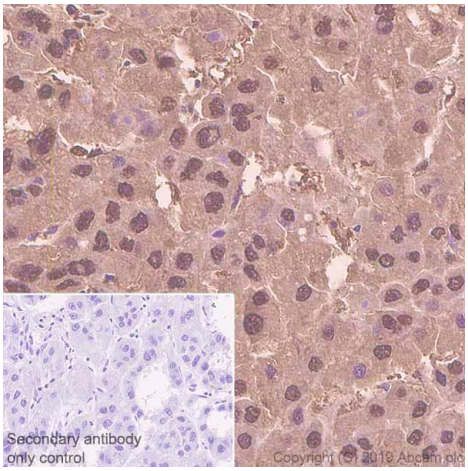
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 30 kDa
Observed band size: 23 kDa

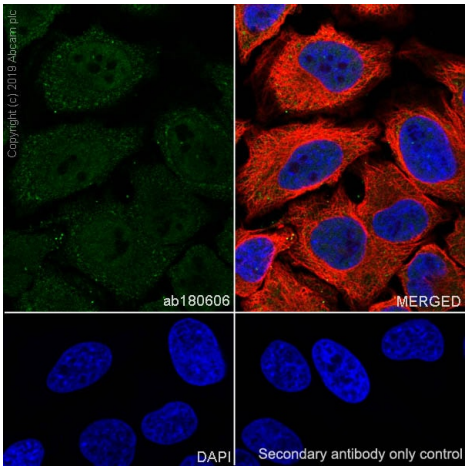
Lanes 1-4: Merged signal (red and green). Green - ab180606 observed at 23 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab180606 Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] was shown to specifically react with Proteasome 20S LMP7 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab267149** (knockout cell lysate **ab257130**) was used. Wild-type and Proteasome 20S LMP7 knockout samples were subjected to SDS-PAGE. ab180606 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



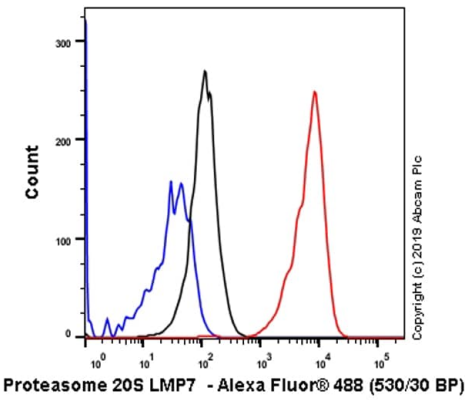
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric carcinoma tissue sections labeling Proteasome 20S LMP7 with purified ab180606 at 1/1700 dilution (0.52 µg/ml). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



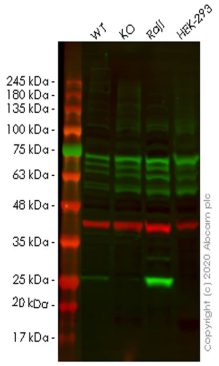
Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Proteasome 20S LMP7 with purified ab180606 at 1:100 dilution (8.9 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Intracellular Flow Cytometry analysis of Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling Proteasome 20S LMP7 with purified ab180606 at 1/90 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

All lanes : Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : PSMB8 knockout A549 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : HEK-293 cell lysate

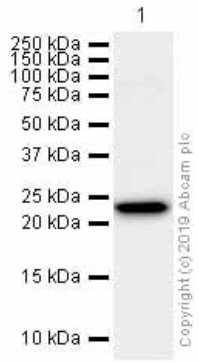
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 30 kDa
Observed band size: 23 kDa

Lanes 1-4: Merged signal (red and green). Green - ab180606 observed at 23 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab180606 Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] was shown to specifically react with Proteasome 20S LMP7 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab267148** (knockout cell lysate **ab257129**) was used. Wild-type and Proteasome 20S LMP7 knockout samples were subjected to SDS-PAGE. ab180606 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

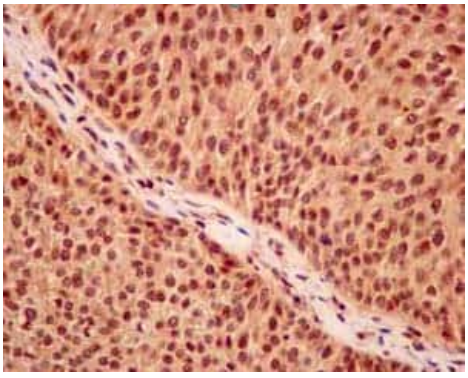


Western blot - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606) at 1/10000 dilution (Purified) + Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysates at 15 µg

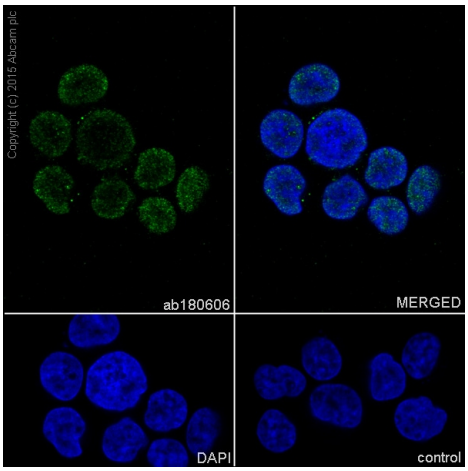
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 30 kDa
Observed band size: 23 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

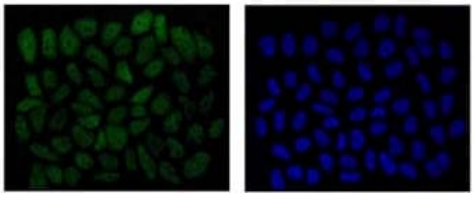
Immunohistochemical analysis of paraffin-embedded human bladder transitional cell carcinoma tissue labeling Proteasome 20S LMP7 with ab180606 (unpurified) at 1/500 dilution, followed by prediluted ImmunoHistoprobe (Ready to use) HRP Polymer for Rabbit IgG. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

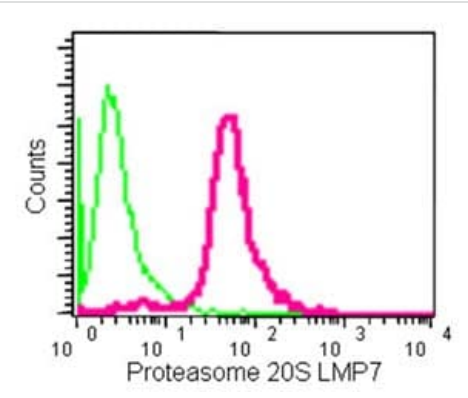
ab180606 (unpurified) staining Proteasome 20S LMP7 in Jurkat (human acute T cell leukemia) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a dilution of 1/1000. DAPI was used as a nuclear counterstain.

Negative control: PBS only.



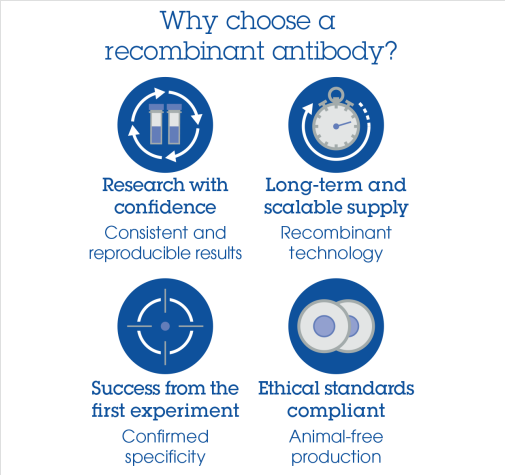
Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Immunofluorecenct analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Proteasome 20S LMP7 with ab180606 (unpurified) at 1/100 dilution, followed by Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody at 1/200 dilution (left panel). DAPI staining (right panel).



Flow Cytometry (Intracellular) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] (ab180606)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixedJurkat (Human T cell leukemia cell line from peripheral blood) cells labeling Proteasome 20S LMP7 with ab180606 (unpurified) at 1/150 dilution (red) compared to a Rabbit monoclonal IgG Isotype control (green), followed byGoat anti rabbit IgG (FITC) secondary antibody at 1/150 dilution.



Anti-Proteasome 20S LMP7 antibody

[EPR14482(B)] (ab180606)

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