abcam

Product datasheet

Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free ab246363

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free
Description	Rabbit monoclonal [EPR14482(B)] to Proteasome 20S LMP7 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF **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	ab246363 is the carrier-free version of **ab180606**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR14482(B)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab246363 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 23 kDa (predicted molecular weight: 30 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0).
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

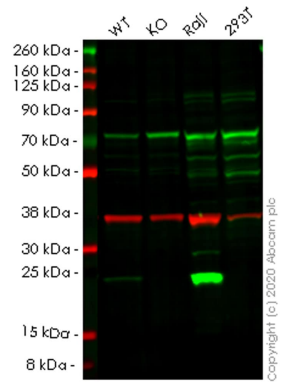
Target

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)] - BSA and Azide free (ab246363)

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

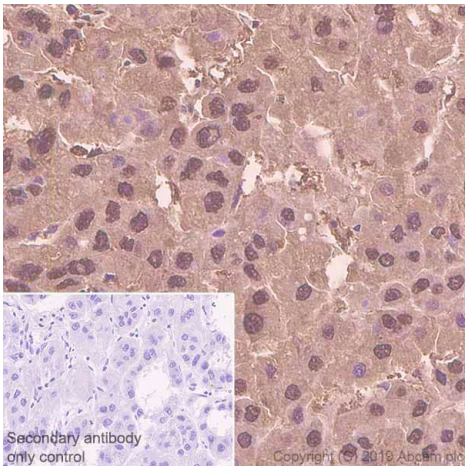
Predicted band size: 30 kDa
Observed band size: 23 kDa

This data was developed using **ab180606**, the same antibody clone in a different buffer formulation.

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

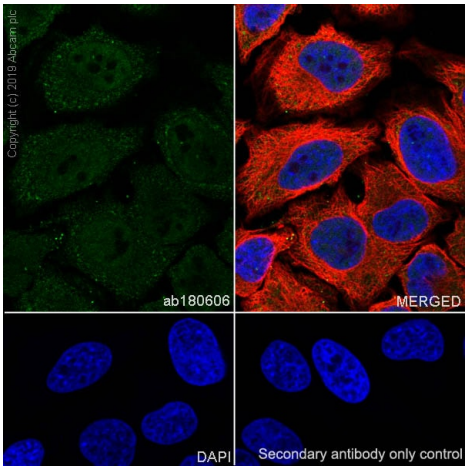
ab180606 Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] was shown to specifically react with Proteasome 20S LMP7 in wild-type A549 cells. Loss of signal was observed when knockout cell

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.



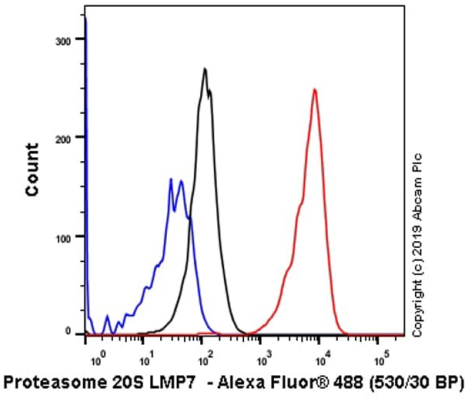
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab180606**)



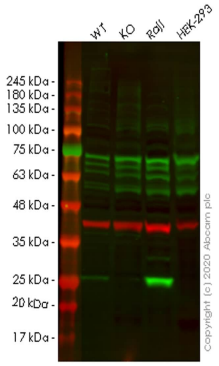
Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (anti proteasome 20s lmp7 antibody epr14482 b immunocytochemistry hela human)



Flow Cytometry (Intracellular) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab180606**)



Western blot - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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

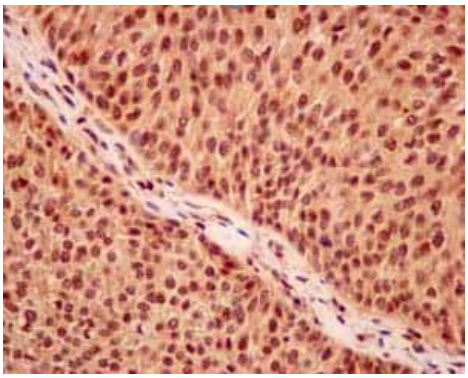
Predicted band size: 30 kDa
Observed band size: 23 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab180606**).

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-

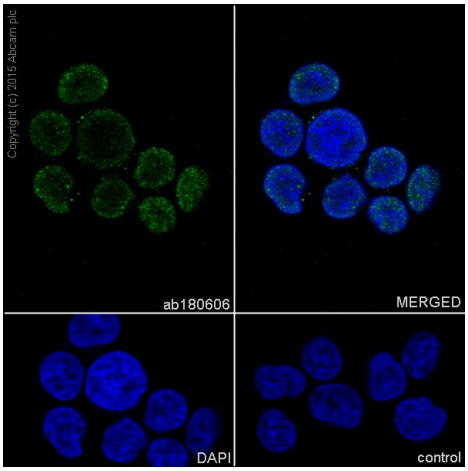
type A549 cells. Loss of signal was observed when knockout cell line **ab267148** (knockout cell lysate **ab257129**) was used. Wild-type and Proteasome 20S LMP7 knockout samples were subjected to SDS-PAGE. **ab180606** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab180606**).



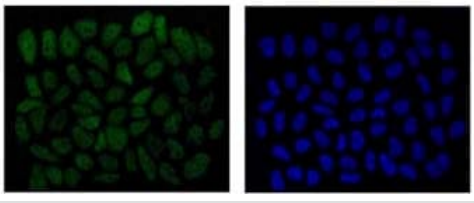
Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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.

This data was developed using the same antibody clone in a

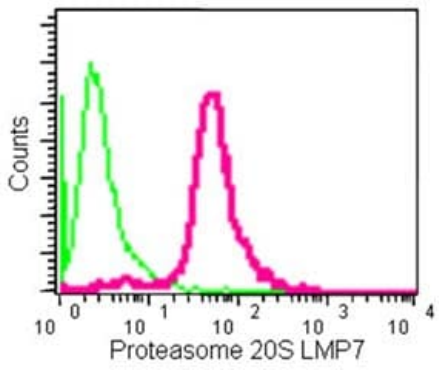
different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab180606**).



Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab180606**).



Flow Cytometry (Intracellular) - Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab180606**).

Anti-Proteasome 20S LMP7 antibody [EPR14482(B)] - BSA and Azide free (ab246363)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors