abcam

Product datasheet

Anti-Proteasome 20S LMP7 antibody ab3329

12 Abreviews 26 References 6 Images

Overview

Product name	Anti-Proteasome 20S LMP7 antibody
Description	Rabbit polyclonal to Proteasome 20S LMP7
Host species	Rabbit
Specificity	Detects proteasome 20S LMP7.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IHC-Fr, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Cow
Immunogen	Synthetic peptide corresponding to Human Proteasome 20S LMP7 aa 259-274. Sequence: VESTDVSDLLHQYREA Database link: P28062 (Peptide available as ab4945) Run BLAST with Run BLAST with

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Constituents: 0.1% BSA, 99% PBS
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab3329** in the following tested applications.

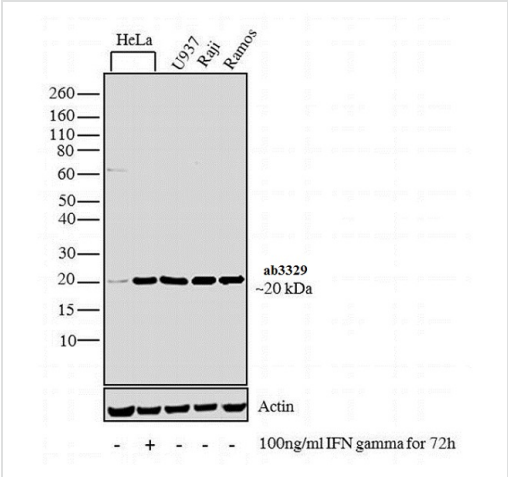
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆	Use a concentration of 1 - 3 µg/ml. Detects a band of approximately 20 kDa.Can be blocked with Human Proteasome 20S LMP7 peptide (ab4945).
IHC-P		Use a concentration of 1 µg/ml.
ICC/IF		Use a concentration of 5 µg/ml.
IHC-Fr		1/500. PubMed: 17008387
Flow Cyt		Use 3-5µg for 10^6 cells.

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 (ab3329)

All lanes : Anti-Proteasome 20S LMP7 antibody (ab3329) at 2 µg/ml

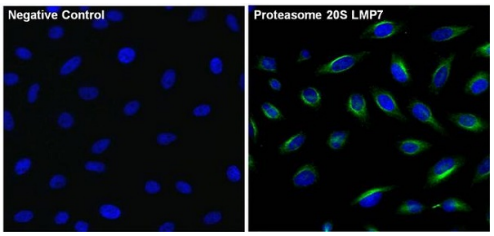
Lane 1 : HeLa whole cell extracts
Lane 2 : HeLa treated with IFN gamma (100ng/ml IFN gamma for 72h) whole cell extracts
Lane 3 : U-937 whole cell extracts
Lane 4 : Raji whole cell extracts
Lane 5 : Ramos whole cell extracts

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG (H+L) HRP conjugate at 1/2500 dilution

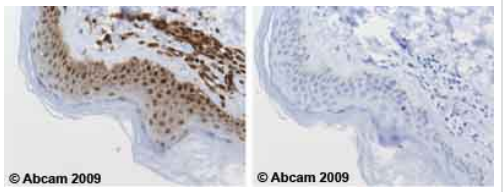
Observed band size: 20 kDa
why is the actual band size different from the predicted?

Detected by chemiluminescence.



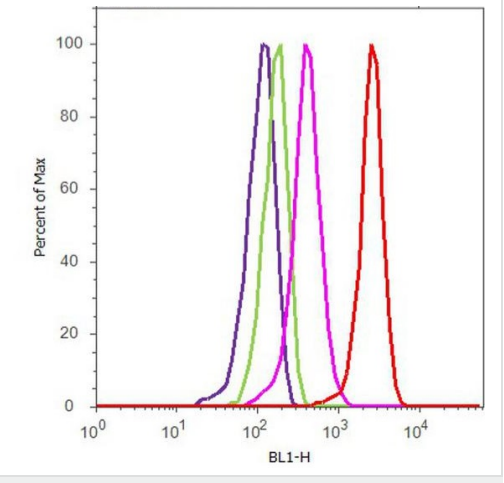
Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody (ab3329)

Immunocytochemistry/ Immunofluorescence analysis of HeLa cells labeling Proteasome 20S LMP7 with ab3329 at 5µg/ml. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton X-100 in TBS for 10 minutes, and blocked with 3% Blocker BSA in PBS for 15 minutes at room temperature. Cells were stained with or without Anti-Proteasome 20S LMP7 antibody (ab3329), at a concentration of 5µg/ml for 1 hour at room temperature, and then incubated with a Alexa Fluor® 488 goat anti-rabbit IgG secondary antibody at a dilution of 1/1000 for 1 hour s at room temperature (both panels, green). Nuclei (both panels, blue) were stained with Hoechst 33342 dye.



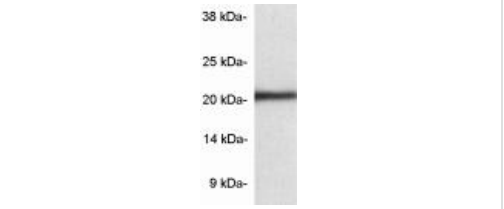
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Proteasome 20S LMP7 antibody (ab3329)

Ab3329 staining Human normal skin. Staining is localized to cytoplasmic and nuclear compartments.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffer citrate pH 6.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS), then incubated with primary antibody for 20 minutes, and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required



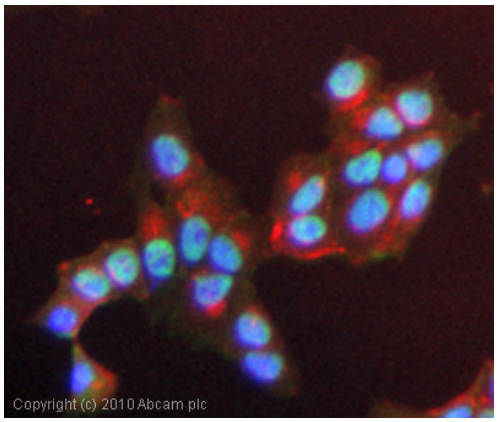
Flow Cytometry - Anti-Proteasome 20S LMP7 antibody (ab3329)

Flow Cytometry analysis of SH-SY5Y cells labeling Proteasome 20S LMP7 with ab3329. Cells were fixed with 70% ethanol for 10 minutes, permeabilized with 0.25% Triton™ X-100 for 20 minutes, and blocked with 5% BSA for 30 minutes at room temperature. Cells were labeled with Anti-Proteasome 20S LMP7 antibody (ab3329) (red histogram) or with rabbit isotype control (pink histogram) at 3-5 ug/million cells in 2.5% BSA. After incubation at room temperature for 2 hours, the cells were labeled with Alexa Fluor® 488 Goat Anti-Rabbit Secondary Antibody at a dilution of 1/400 for 30 minutes at room temperature. The representative 10,000 cells were acquired and analyzed for each sample. The purple histogram represents unstained control cells and the green histogram represents no-primary-antibody control.



Western blot - Anti-Proteasome 20S LMP7 antibody (ab3329)

Western blot of proteasome 20S LMP7 from HeLa cell extract using ab3329.



Immunocytochemistry/ Immunofluorescence - Anti-Proteasome 20S LMP7 antibody (ab3329)

ICC/IF image of ab3329 stained MCF7 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab3329, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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