abcam

Product datasheet

Human PSMB8 (Proteasome 20S LMP7) knockout A549 cell line ab267148

4 Images

Overview

Product name	Human PSMB8 (Proteasome 20S LMP7) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type A549 cell line (**ab255450**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** F-12K + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^3$-$1x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $6x10^4$ cells/cm^2 is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
Do not exceed $7x10^4$ cells/cm^2.
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We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 vWA: 14 TH01: 8,9.3 TPOX: 8,11 CSF1PO: 10, 12
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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	Autocleaved. The resulting N-terminal Thr residue of the mature subunit is responsible for the

modifications	nucleophile proteolytic activity.
Cellular localization	Cytoplasm. Nucleus.

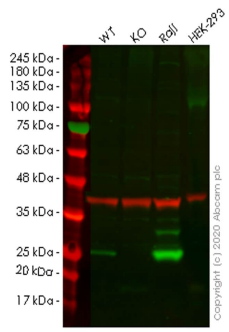
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab267148 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 at an assay dependent concentration. Predicted molecular weight: 30 kDa.

Images



Western blot - Human PSMB8 (Proteasome 20S LMP7) knockout A549 cell line (ab267148)

All lanes : Anti-Proteasome 20S LMP7 antibody (**ab82528**) at 1/500 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.

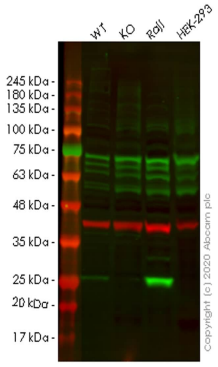
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

Lanes 1-4: Merged signal (red and green). Green - **ab82528** observed at 23 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab82528 Anti-Proteasome 20S LMP7 antibody 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. **ab82528** and Anti-GAPDH antibody [6C5] - Loading

Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 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 - Human PSMB8 (Proteasome 20S LMP7) knockout A549 cell line (ab267148)

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

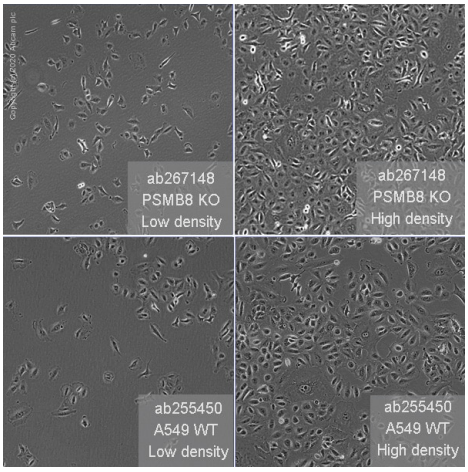
Predicted band size: 30 kDa
Observed band size: 23 kDa

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.

Sanger Sequencing - Human PSMB8 knockout A549 cell line (ab267148)

Homozygous: 1 bp insertion in exon3



Cell Culture - Human PSMB8 (Proteasome 20S LMP7) knockout A549 cell line (ab267148)

Representative images of PSMB8 knockout A549 cells, low and high confluency examples (top left and right respectively) and wild-type A549 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

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