abcam

Product datasheet

Human BSG (CD147) knockout A549 cell line ab273748

11 图像

概述

产品名称	人BSG (CD147) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 78 bp insertion in exon 5 introducing premature STOP codon
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB, Flow Cyt, Sandwich ELISA
Biosafety level	1
常规说明	**Recommended control:** Human wild-type A549 cell line (**ab275463**). 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.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Plays pivotal roles in spermatogenesis, embryo implantation, neural network formation and tumor progression. Stimulates adjacent fibroblasts to produce matrix metalloproteinases (MMPS). May target monocarboxylate transporters SLC16A1, SLC16A3 and SLC16A8 to plasma membranes of retinal pigment epithelium and neural retina. Seems to be a receptor for oligomannosidic glycans. In vitro, promotes outgrowth of astrocytic processes.
组织特异性	Present only in vascular endothelium in non-neoplastic regions of the brain, whereas it is present in tumor cells but not in proliferating blood vessels in malignant gliomas.
序列相似性	Contains 1 Ig-like C2-type (immunoglobulin-like) domain. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
翻译后修饰	N-glycosylated.
细胞定位	Cell membrane. Melanosome. Colocalizes with SLC16A1 and SLC16A8 (By similarity). Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

应用

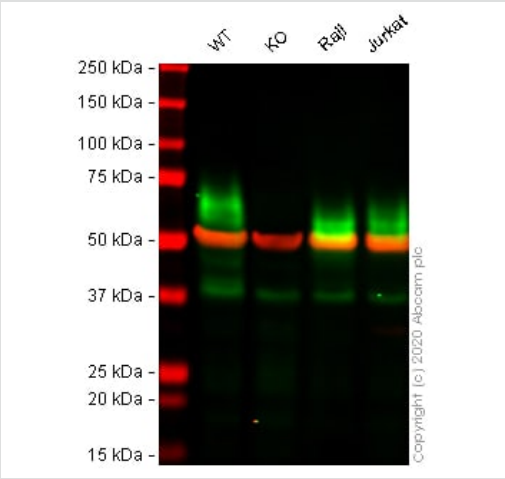
The Abpromise guarantee **Abpromise™承诺保证使用**ab273748**于以下的**经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 42 kDa.
Flow Cyt		Use at an assay dependent concentration.
Sandwich ELISA		Use at an assay dependent concentration.

图片



Western blot - Human BSG (CD147) knockout A549 cell line (ab273748)

All lanes : Anti-CD147 antibody [MEM-M6/1] (**ab666**) at 1 µg/ml

Lane 1 : Wild-type A549 cell lysate
Lane 2 : BSG knockout A549 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Jurkat cell lysate

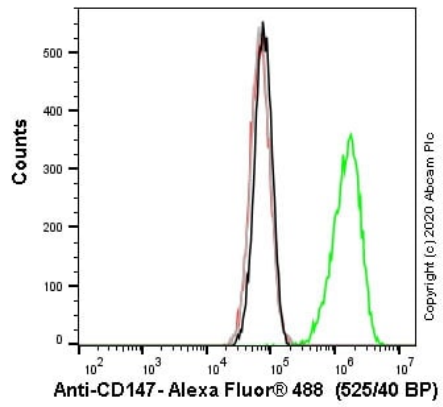
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 55-70 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab666** observed at 55-70 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab666 was shown to react with CD147 in wild-type A549 cells in western blot with loss of signal observed in BSG knockout cell line ab273748 (knockout cell lysate **ab275500**). Wild-type and BSG knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab666** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



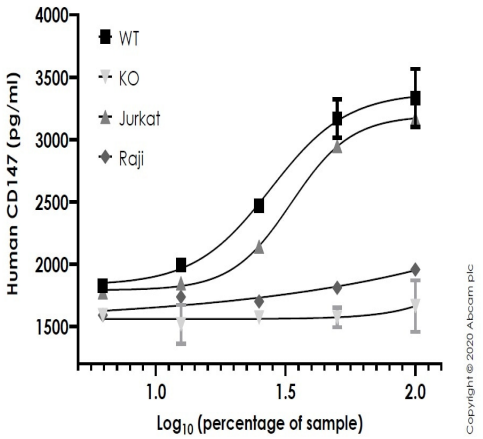
Flow Cytometry (Intracellular) - Human BSG (CD147) knockout A549 cell line (ab273748)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (ab273748) stained with **ab666** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab666**) ($1x10^6$ in 100µl at 10 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

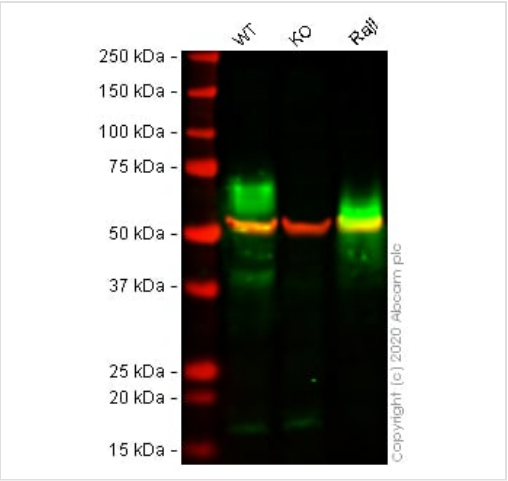
Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Sandwich ELISA - Human BSG (CD147) knockout A549 cell line (ab273748)

Human CD147 concentration was interpolated from the EMMPRIN (CD147) standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human EMMPRIN ELISA kit (**ab219631**). Wild-type and CD147 knockout A549 cells (ab273748) were assessed in duplicate (n=2); Jurkat and Raji cells were used as positive and negative controls respectively (n=1). Where samples were run in duplicate, data are represented as the mean and error bars represent standard deviation.



Western blot - Human BSG (CD147) knockout A549 cell line (ab273748)

All lanes : Anti-CD147 antibody [EPR4053] (**ab108308**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : BSG knockout A549 cell lysate
Lane 3 : Raji cell lysate

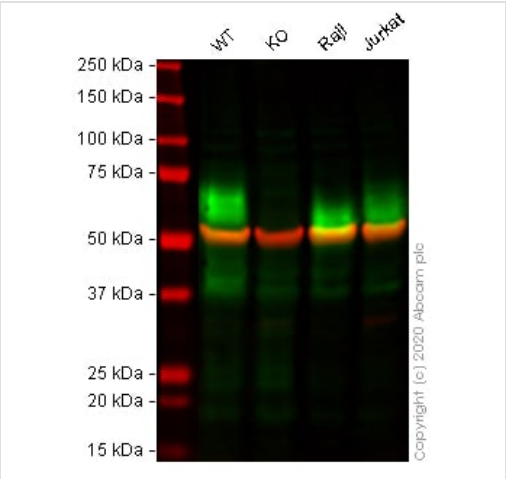
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 42-70 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab108308** observed at 42-70 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab108308 was shown to react with CD147 in wild-type A549 cells in western blot with loss of signal observed in BSG knockout cell line ab273748 (knockout cell lysate **ab275500**). Wild-type and BSG knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab108308** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human BSG (CD147) knockout A549 cell line (ab273748)

All lanes : Anti-CD147 antibody [OTI9B10] (**ab119020**) at 1/2000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : BSG knockout A549 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Jurkat cell lysate

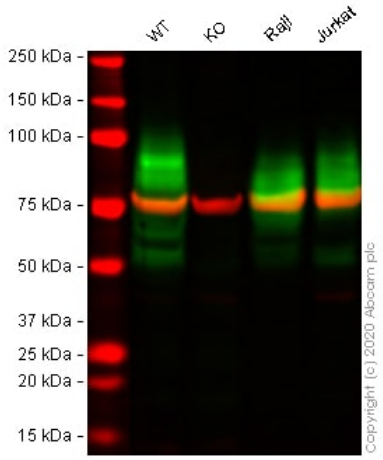
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 55-70 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab119020** observed at 55-70 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab119020 was shown to react with CD147 in wild-type A549 cells in western blot with loss of signal observed in BSG knockout cell line ab273748 (knockout cell lysate **ab275500**). Wild-type and BSG knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab119020** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 2000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human BSG (CD147) knockout A549 cell line (ab273748)

All lanes : Anti-CD147 antibody [10E10] (**ab230921**) at 1/500 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : BSG knockout A549 cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Jurkat cell lysate

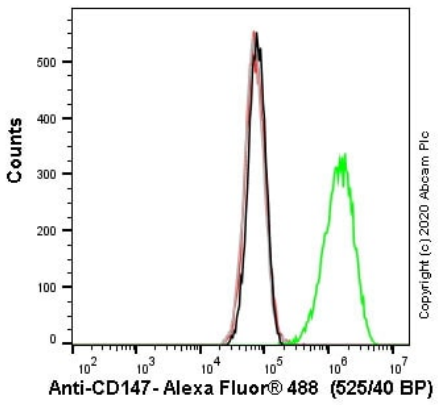
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 42-70 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab230921** observed at 42-70 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab230921 was shown to react with CD147 in wild-type A549 cells in western blot with loss of signal observed in BSG knockout cell line ab273748 (knockout cell lysate **ab275500**). Wild-type and BSG knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab230921** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 500 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



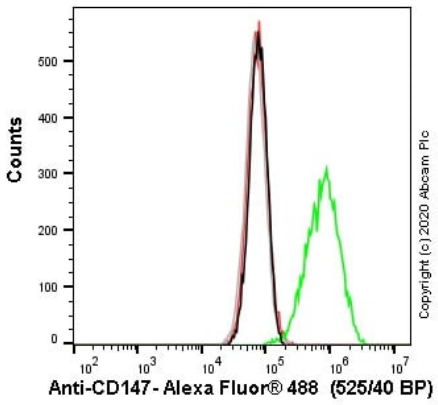
Flow Cytometry - Human BSG (CD147) knockout A549 cell line (ab273748)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (ab273748) stained with **ab194401** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab194401**) ($1x10^6$ in 100µl at 10 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



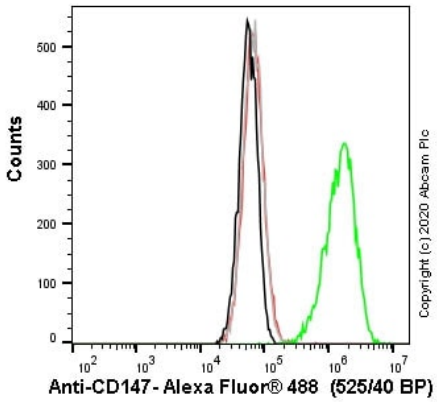
Flow Cytometry - Human BSG (CD147) knockout A549 cell line (ab273748)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (ab273748) stained with **ab91147** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab91147**) ($1x10^6$ in 100µl at 10 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



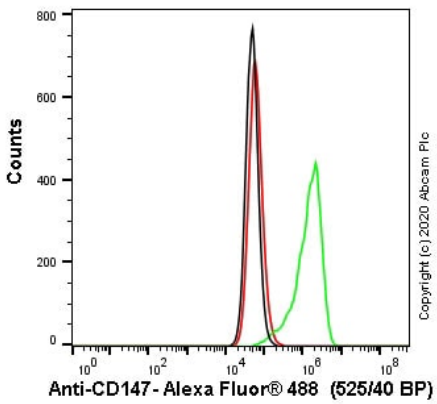
Flow Cytometry - Human BSG (CD147) knockout A549 cell line (ab273748)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (ab273748) stained with **ab21903** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab21903**) ($1x10^6$ in 100µl at 5 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was mouse IgG2bκ (**ab170192**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



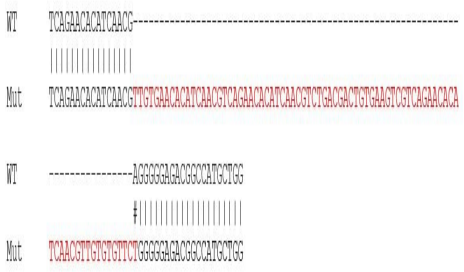
Flow Cytometry - Human BSG (CD147) knockout A549 cell line (ab273748)

Flow cytometry overlay histogram showing wild-type A549 (green line) and BSG knockout A549 cells (ab273748) stained with **ab230921** (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab230921**) ($1x10^6$ in 100µl at 5 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A549 - black line; BSG knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Sanger Sequencing - Human BSG (CD147) knockout A549 cell line (ab273748)

Allele-1: 78 bp insertion in exon 5 introducing premature STOP codon.

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