abcam

Product datasheet

Human CD74 knockout Raji cell line ab273378

10 图像

概述

产品名称	人CD74 knockout Raji cell line
Parental Cell Line	Raji
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 13 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
经测试应用	**适用于:** WB, Flow Cyt, Flow Cyt (Intra)
Biosafety level	2
常规说明	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended control:** Human wild-type Raji cell line (**ab275473**). 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:** RPMI + 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 for bath 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 $4x10^5$ cells/mL. 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. **Initial handling guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $4x10^5$ cells/mL is recommended.

A maximum of $3x10^6$ viable cells/ml is obtainable.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant 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	Suspension
Tissue	Lymphatic
Cell type	Burkitt's lymphoma
Disease	Lymphoma
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 a critical role in MHC class II antigen processing by stabilizing peptide-free class II alpha/beta heterodimers in a complex soon after their synthesis and directing transport of the complex from the endoplasmic reticulum to the endosomal/lysosomal system where the antigen processing and binding of antigenic peptides to MHC class II takes place. Serves as cell surface receptor for the cytokine MIF.
序列相似性	Contains 1 thyroglobulin type-1 domain.
细胞定位	Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus > trans-Golgi network. Endosome. Lysosome. Transits through a number of intracellular compartments in the endocytic pathway. It can either undergo proteolysis or reach the cell membrane.

应用

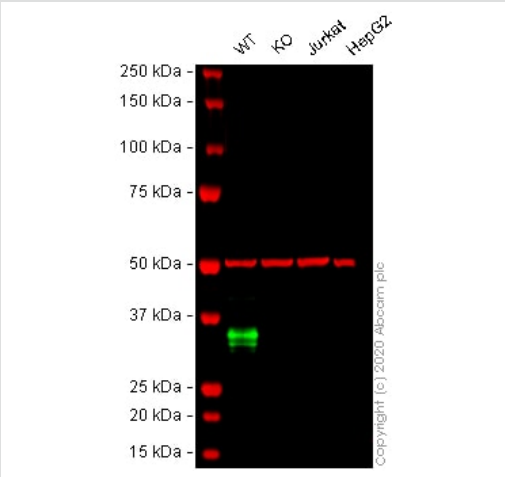
The Abpromise guarantee **Abpromise™承诺保证使用ab273378于以下的经测试应用**

"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
WB		Use at an assay dependent concentration. Predicted molecular weight: 34 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Flow Cyt		Use at an assay dependent concentration.

应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration.

图片



Western blot - Human CD74 knockout Raji cell line (ab273378)

All lanes : Anti-CD74 antibody [CLIP/3127R] (**ab270265**) at 1 µg/ml

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

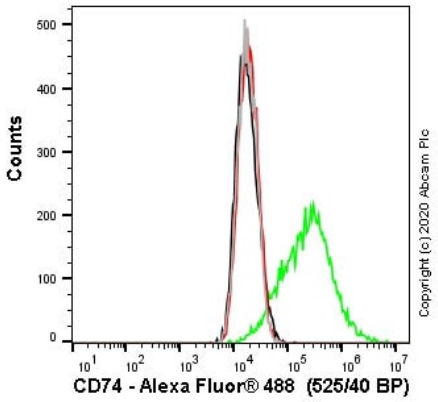
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab270265** observed at 35 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab270265 was shown to react with CD74 in western blot. The band observed in CD74 knockout cell line ab273378 (knockout lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab270265** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at 1 µg/ml 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 h at room temperature before imaging.



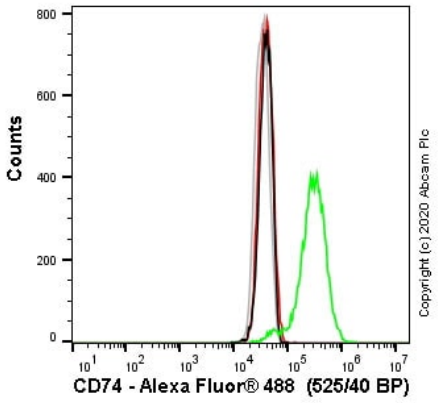
Flow Cytometry - Human CD74 knockout Raji cell line (ab273378)

Flow cytometry overlay histogram showing wild-type Raji (green line) and CD74 knockout Raji cells (ab273378) stained with **ab270265** (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab270265**) ($1x10^6$ in 100µl at 1 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type Raji cells - black line; CD74 knockout Raji cells ab273378 - 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.



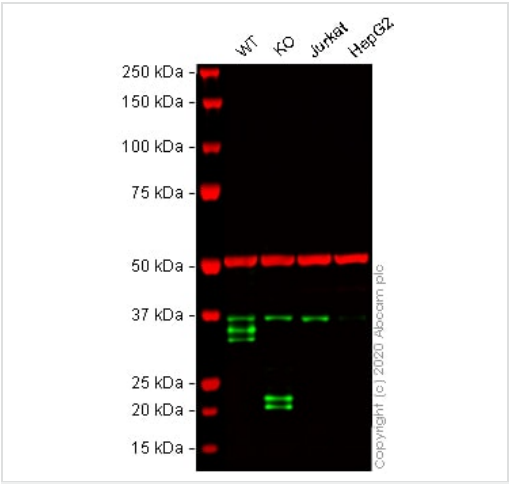
Flow Cytometry (Intracellular) - Human CD74 knockout Raji cell line (ab273378)

Flow cytometry overlay histogram showing wild-type Raji (green line) and CD74 knockout Raji cells (ab273378) stained with **ab108393** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab108393**) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type Raji cells - black line; CD74 knockout Raji cells ab273378 - 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.



Western blot - Human CD74 knockout Raji cell line (ab273378)

All lanes : Anti-CD74 antibody [EPR4064] (**ab108393**) at 1/1000 dilution

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

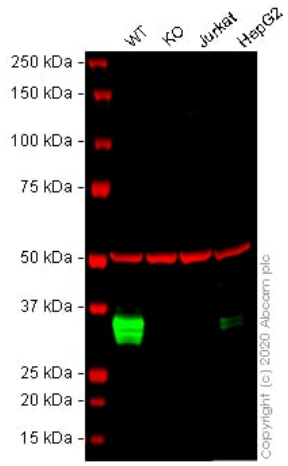
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab108393** observed at 35 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab108393 was shown to react with CD74 in western blot. The band observed in CD74 knockout cell line ab273378 (knockout lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab108393** 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 h at room temperature before imaging.



Western blot - Human CD74 knockout Raji cell line (ab273378)

All lanes : Anti-CD74 antibody (**ab64772**) at 1 µg/ml

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

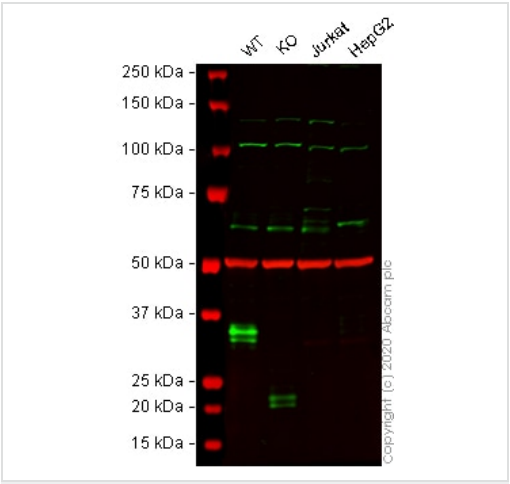
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab64772** observed at 35 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab64772 was shown to react with CD74 in western blot. The band observed in CD74 knockout cell line ab273378 (knockout lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab64772** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at 1 µg/ml 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 h at room temperature before imaging.



Western blot - Human CD74 knockout Raji cell line (ab273378)

All lanes : Anti-CD74 antibody [PIN.1] (**ab22603**) at 1 µg/ml

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

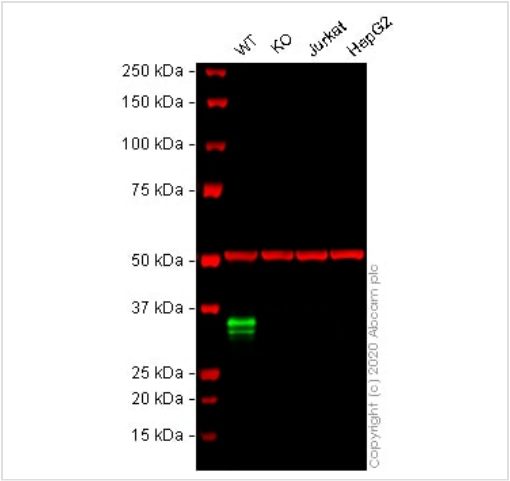
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab22603** observed at 35 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab22603 was shown to react with CD74 in western blot. The band observed in CD74 knockout cell line ab273378 (knockout lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab22603** 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 h at room temperature before imaging.



Western blot - Human CD74 knockout Raji cell line (ab273378)

All lanes : Anti-CD74 antibody [LN2] (**ab9514**) at 5 µg/ml

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

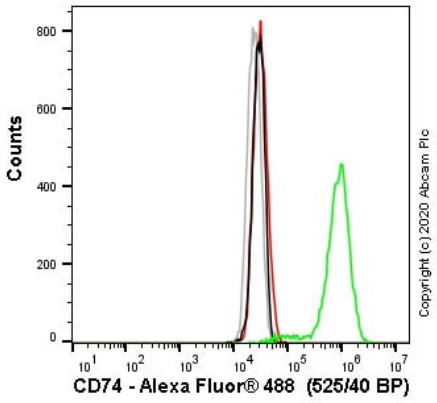
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab9514** observed at 35 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab9514 was shown to react with CD74 in western blot. The band observed in CD74 knockout cell line ab273378 (knockout lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab9514** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 °C at 5 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 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Human CD74 knockout Raji cell line (ab273378)

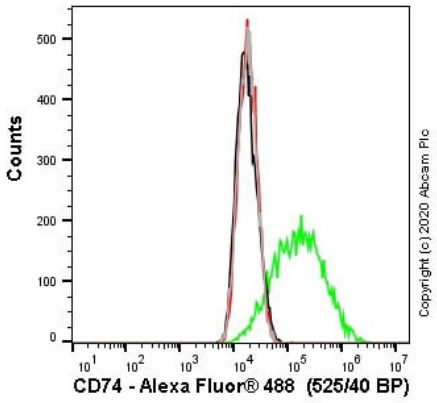
Flow cytometry overlay histogram showing wild-type Raji (green line) and CD74 knockout Raji cells (ab273378) stained with **ab9514** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab9514**) ($1x10^6$ in 100µl at 1 µg/ml) for 30 min at 22°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 22°C.

Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type Raji cells - black line; CD74 knockout Raji cells ab273378 - 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.

This antibody gave a positive signal in CD74 knockout Raji cells fixed with 4% formaldehyde (10 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



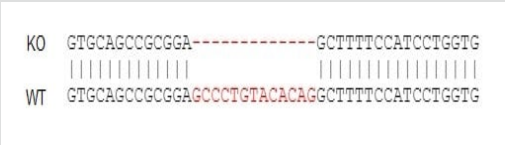
Flow Cytometry - Human CD74 knockout Raji cell line (ab273378)

Flow cytometry overlay histogram showing wild-type Raji (green line) and CD74 knockout Raji cells (ab273378) stained with **ab22606** (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab22606**) ($1x10^6$ 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 Raji cells - black line; CD74 knockout Raji cells ab273378 - 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 CD74 knockout Raji cell line (ab273378)

Homozygous: 13 bp deletion in exon 2

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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