abcam

Product datasheet

Anti-CD74 antibody ab64772

KO 評価済

★★★★★ 1 Abreviews 4 References 画像数 8

製品の概要

製品名	Anti-CD74 antibody
製品の詳細	Rabbit polyclonal to CD74
由来種	Rabbit
アプリケーション	**適用あり:** WB, ICC/IF, IP, IHC-P
種交差性	**交差種:** Human **交差が予測される動物種:** Non human primates
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Raji and Daudi cell lysates. IF/ICC: Raw246.7 cell line. IHC-P: Human tonsil and lymph node tissues. IP: Raji whole cell extract.
特記事項	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Immunogen affinity purified

ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab64772の使用に適用されます

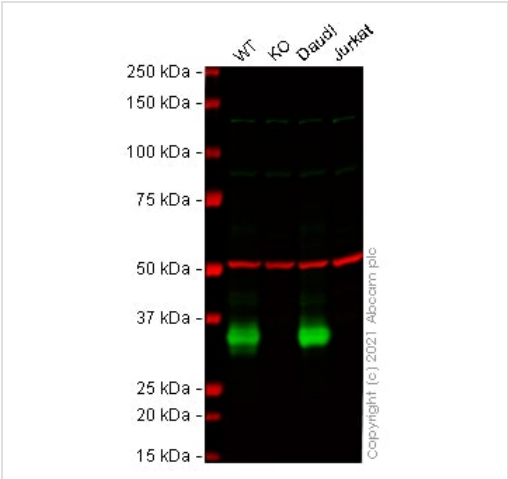
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
ICC/IF		Use a concentration of 1 µg/ml.
IP		Use a concentration of 5 µg/ml. IP image from Phase V. Lot JLD 04.06.2013
IHC-P	★★★★★ (1)	1/80 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ターゲット情報

機能	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.
配列類似性	Contains 1 thyroglobulin type-1 domain.
細胞内局在	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.

画像



Western blot - Anti-CD74 antibody (ab64772)

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

Lane 1 : Wild-type Raji (Human Burkitt's lymphoma cell line) whole cell lysate
Lane 2 : CD74 knockout Raji (Human Burkitt's lymphoma cell line) whole cell lysate
Lane 3 : Daudi (Human Burkitt's lymphoma cell line) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

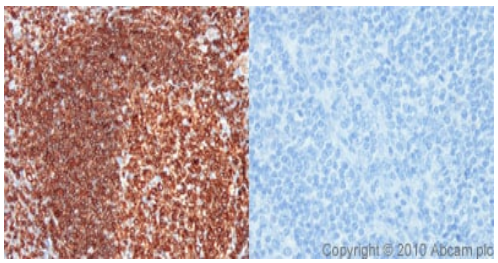
Performed under reducing conditions.

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 wild-type Raji cells in Western blot with loss of signal observed in CD74 knockout cell line **ab273876** (knockout cell lysate **ab273830**). Wild-type Raji and CD74 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) 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

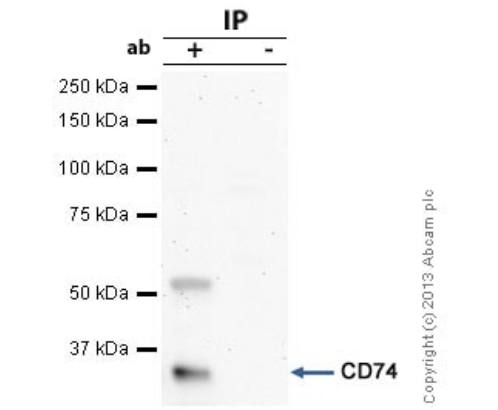
temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody (ab64772)

ab64772 (1:160) staining CD74 in paraffin-embedded human tonsil (left panel) using an automated system (Ventana Discovery). Right-hand panel shows negative control (no primary antibody).
Using this protocol there is strong membrane staining of activated B cells in the germinal centres and B cells of the mantle zone of the follicles plus scattered cells of the interfollicular areas (paracortical B cells).

Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Mild Retrieval programme. Slides were blocked in 3% H_2O_2 / 4 min / 37°C and incubated with ab64772 (1:160 dilution / 2 hours / 37°C). Sections then blocked (4mins / 37°C) and incubated with Dako swine anti-rabbit antibody (1:50, 28 min / 37°C). Staining was amplified and detected by incubation with Ventana Streptavidin ABC system (16 min / 37°C) and Ventana DAB map reagent (8 min / 37°C). Slides were counterstained with Haematox



Immunoprecipitation - Anti-CD74 antibody (ab64772)

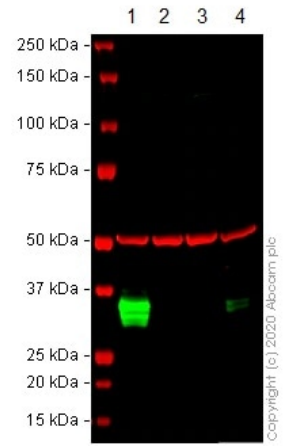
CD74 was immunoprecipitated using 0.5mg Raji whole cell extract, 5µg of Rabbit polyclonal to CD74 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Raji whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab64772.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 34kDa; CD74



Western blot - Anti-CD74 antibody (ab64772)

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

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 CRISPR/Cas9 edited 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 CRISPR/Cas9 edited cell line **ab273378** (CRISPR/Cas9 edited 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 - Anti-CD74 antibody (ab64772)

Anti-CD74 antibody (ab64772) at 1 µg/ml + Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate at 10 µg

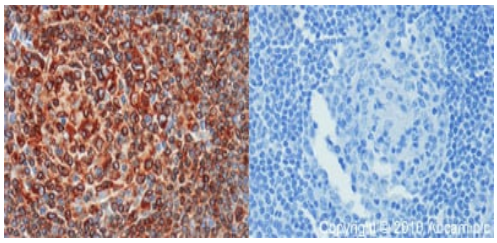
Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

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

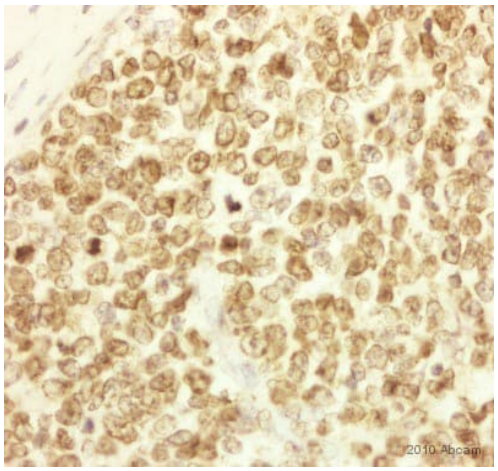
Exposure time: 2 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody (ab64772)

ab64772 (1:80) staining CD74 in paraffin-embedded human lymph node (left panel) using an automated system (Ventana Discovery). Right-hand panel shows negative control (no primary antibody). Using this protocol there is strong membrane staining of activated B cells in the germinal centres and B cells of the mantle zone of the follicles plus scattered cells of the interfollicular areas (paracortical B cells).

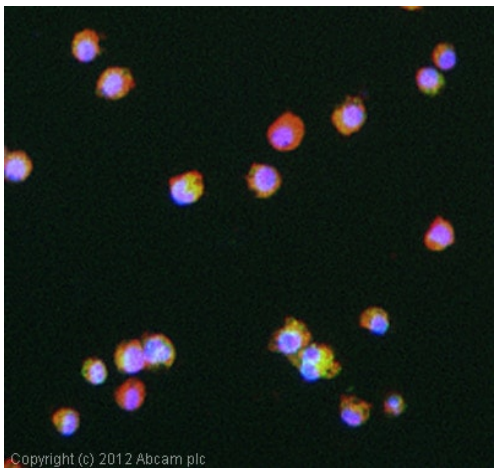
Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Mild Retrieval programme. Slides were blocked in 3% H_2O_2 / 4 min / 37°C and incubated with ab64772 (1:80 dilution / 1 hour / 37°C). Sections then blocked (3mins / 37°C) and incubated with Dako swine anti-rabbit antibody (1:50, 28 min / 37°C). Staining was amplified and detected by incubation with Ventana Streptavidin ABC system (16 min / 37°C) and Ventana DAB map reagent (8 min / 37°C). Slides were counterstained with Haematoxyli



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody (ab64772)

This image is courtesy of an abreview submitted by Antibody Solutions Ltd.

ab64772 (1/250) staining CD74 in paraffin-embedded Human tonsil tissue. Tissue underwent fixation in formaldehyde, peroxidase blocking, protein blocking and heat mediated antigen retrieval. The secondary antibody was goat anti rabbit conjugated to HRP. For further experimental details please refer to abreview.



Immunocytochemistry/ Immunofluorescence - Anti-CD74 antibody (ab64772)

ICC/IF image of ab64772 stained RAW246.7 cells. The cells were 4% paraformaldehyde 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 (ab64772, 1µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 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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