abcam

Product datasheet

Anti-CD74 antibody ab64772

KO VALIDATED

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Overview

Product name	Anti-CD74 antibody
Description	Rabbit polyclonal to CD74
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Non human primates
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	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

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	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.
Purity	Immunogen affinity purified

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab64772 in the following tested applications.

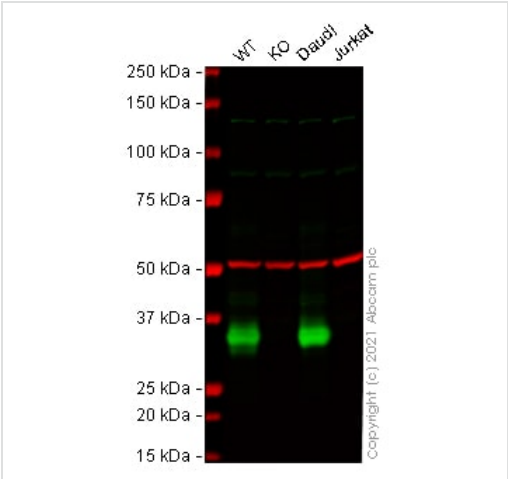
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 1 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.

Target

Function	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.
Sequence similarities	Contains 1 thyroglobulin type-1 domain.
Cellular localization	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.

Images



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.

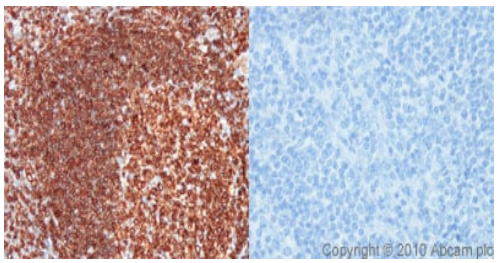
Performed under reducing conditions.

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

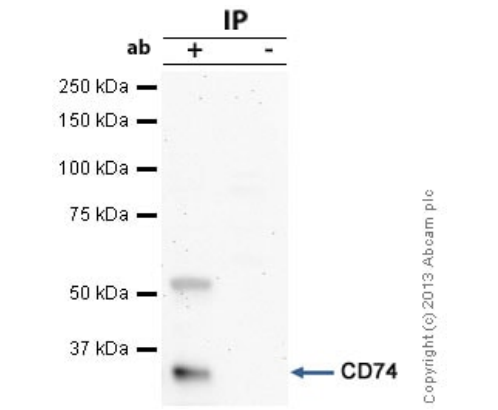
temperature before imaging.



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)

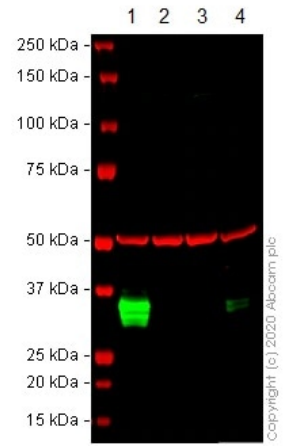
CD74 was immunoprecipitated using 0.5mg Raji whole cell extract, 5µg of Rabbit polyclonal to CD74 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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

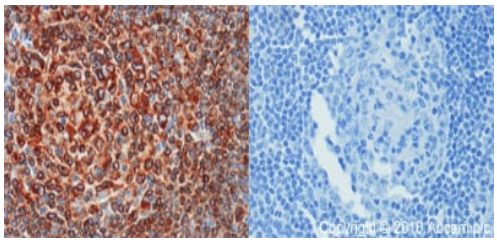
Secondary

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

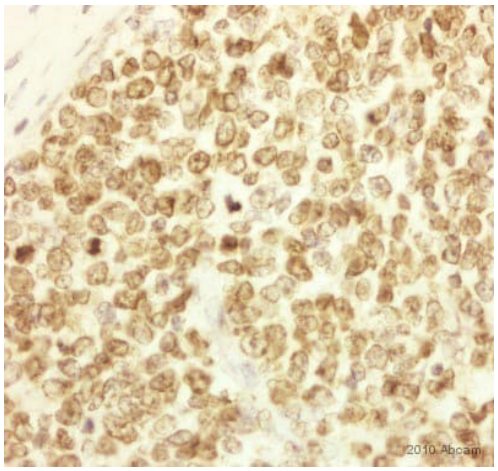
Exposure time: 2 minutes



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).

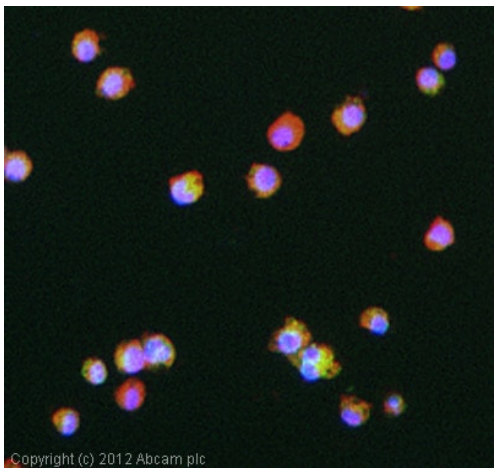
Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Mild Retrieval programme. Slides were blocked in 3% H_2O_2 / 4 min / 37°C and incubated with ab64772 (1:80 dilution / 1 hour / 37°C). Sections then blocked (3mins / 37°C) and incubated with Dako swine anti-rabbit 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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