abcam

Product datasheet

Anti-CD74 antibody ab64772

★★★★★ 1 Abreviews 6 Images

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 conjugated to KLH derived from within residues 250 to the C-terminus of Human CD74.Read Abcam's proprietary immunogen policy(Peptide available as ab74386.)
Positive control	This antibody gave a positive signal in Raji Whole Cell Lysate IF/ICC: Raw246.7 cell line

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	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

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.

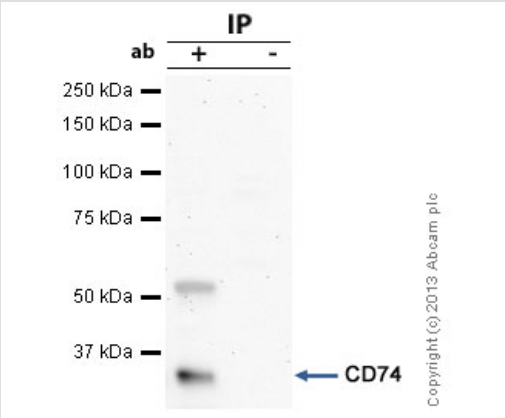
Application	Abreviews	Notes
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

Application	Abreviews	Notes
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	★★★★★	1/80 - 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



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)

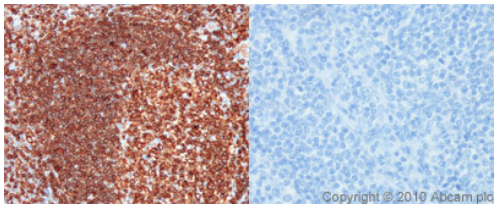
Anti-CD74 antibody (ab64772) at 1 µg/ml + Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate at 10 µg

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

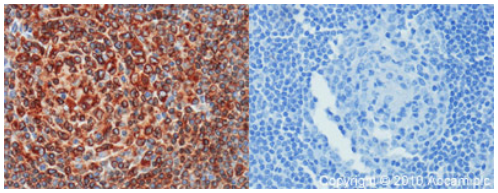
Exposure time: 2 minutes



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

ab64772 (1:160) 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).

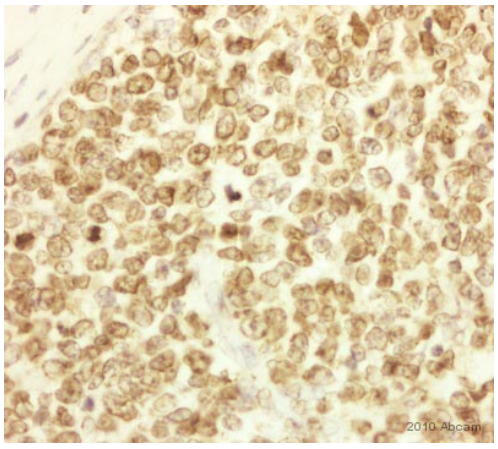
Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Mild Retrieval programme. Slides were blocked in 3% H_2O_2 / 4 min / 37°C and incubated with ab64772 (1:160 dilution / 2 hours / 37°C). Sections then blocked (4mins / 37°C) and incubated with Dako swine anti-rabbit 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



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).

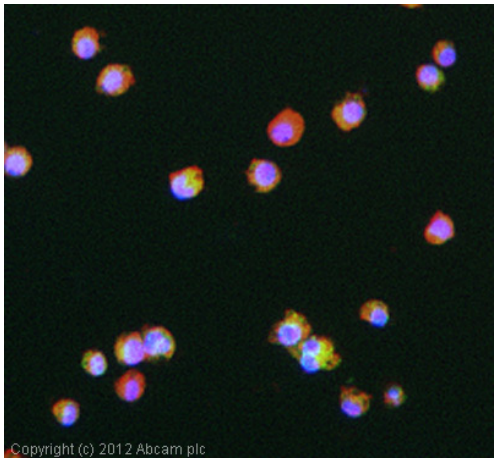
Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Mild Retrieval programme. Slides were blocked in 3% H_2O_2 / 4 min / 37°C and incubated with ab64772 (1:80 dilution / 1 hour / 37°C). Sections then blocked (3mins / 37°C) and incubated with Dako swine anti-rabbit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