abcam

ab9514

Anti-CD74 antibody [LN2]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.3 - 7.5 Preservative: 0.1% Sodium azide Constituents: 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	Cell preparation containing SU-DHL-4 cell line protein. The exact immunogen used to generate this antibody is proprietary information.
Clone number	LN2
Purification technique	Affinity purification Protein A/G
Light chain type	kappa
Concentration	0.05 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/25 - 1/50
Notes	

Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Flow Cyt

Tested

Species	Human
Dilution info	1/10
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	5 µg/mL
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Target data

See full target information CD74

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. Class-II-associated invariant chain peptide. Binds to the peptide-binding site of MHC class II alpha/beta heterodimers forming an alpha-beta-CLIP complex, thereby preventing the loading of antigenic peptides to the MHC class II complex until its release by HLA-DM in the endosome. Isoform p41. Stabilizes the conformation of mature CTSL by binding to its active site and serving as a

chaperone to help maintain a pool of mature enzyme in endocytic compartments and extracellular space of antigen-presenting cells (APCs). Has antiviral activity by stymieing the endosomal entry of Ebola virus and coronaviruses, including SARS-CoV-2 (PubMed:32855215). Disrupts cathepsin-mediated Ebola virus glycoprotein processing, which prevents viral fusion and entry. This antiviral activity is specific to p41 isoform (PubMed:32855215).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD74 also known as the invariant chain or Ii is a protein with a mass of about 33 kDa. It is widely expressed on the surface of immune cells such as B cells dendritic cells and macrophages. CD74 plays an important role in the immune system by acting as a chaperone for major histocompatibility complex class II (MHC II) molecules guiding their migration to endosomes where they encounter antigenic peptides. The CD74 protein also functions as a cell surface receptor for macrophage migration inhibitory factor (MIF) enhancing the immune response process.
Biological function summary	CD74 facilitates multiple immune regulatory processes. It forms a complex with the MHC II molecules aiding in their proper folding and peptide loading in the endoplasmic reticulum. This complex then travels through the Golgi apparatus towards the lysosomal compartments where antigens are presented to initiate adaptive immune responses. In addition CD74 is involved in signal transduction pathways that regulate cell proliferation and survival influenced by interactions with MIF and other molecules.
Pathways	The role of CD74 extends into both the antigen presentation and MIF signaling pathways. Within the antigen presentation pathway CD74's activity is tightly connected with MHC II and subsequently to CD4+ T cells facilitating the activation of these immune cells. The MIF signaling pathway involves CD74 interacting with CD44 a receptor that further propagates the signaling cascades essential for immune modulation and cell survival.

Associated diseases and disorders	CD74 is linked to inflammatory and autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus. Aberrant expression of CD74 influences the presentation of autoantigens and may contribute to the chronic inflammation seen in these disorders. Its connection to MIF which is a pro-inflammatory cytokine further implicates CD74 in these autoimmune scenarios by promoting persistent immune activation. Researchers are also exploring CD74 as a therapeutic target in certain cancers due to its influence on immune surveillance and tumor progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images



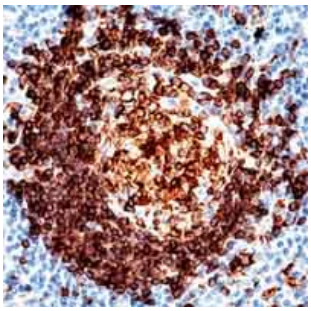
Image courtesy of an anonymous customer review.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [LN2] (ab9514)

CD74 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-CD74 antibody

ab9514 staining CD74 in human liver tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with neutral buffered formalin and a heat mediated antigen retrieval step was performed using EDTA buffer pH 9.0. Samples were then blocked with 10% serum for 10 minutes at 20°C followed by incubation with the primary antibody at a 1/75

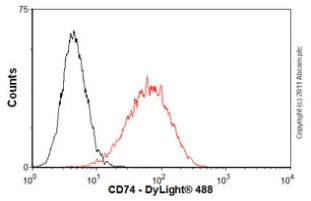
dilution for 30 minutes at 20°C. A HRP-conjugated rat anti-mouse/rabbit polyclonal was used undiluted as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [LN2] (ab9514)

CD74 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-CD74 antibody

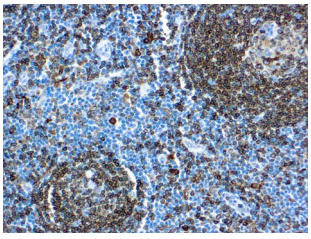
ab9514 staining CD74 in paraffin embedded Human tonsil tissue sections by Immunohistochemistry (IHC-P).



Flow Cytometry - Anti-CD74 antibody [LN2] (ab9514)

CD74 Flow Cytometry staining using mouse Anti-CD74 antibody

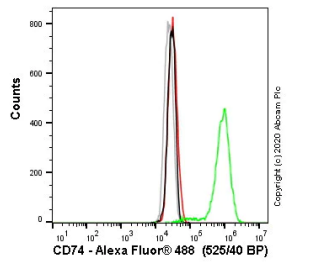
Overlay histogram showing Raji cells stained with ab9514 (red line). The cells were fixed with 80% methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab9514, 1/10 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
Please note that Abcam do not have any data for use of this antibody on non-fixed cells. We welcome any customer feedback.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [LN2] (ab9514)

CD74 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-CD74 antibody

Formalin-fixed, paraffin-embeded human tonsil tissue stained for CD74 using ab9514 at 1/50 dilution in immunohistochemical analysis. Antigen retrieval with citrate buffer pH 6.0.



Flow Cytometry (Intracellular) - Anti-CD74 antibody [LN2] (ab9514)

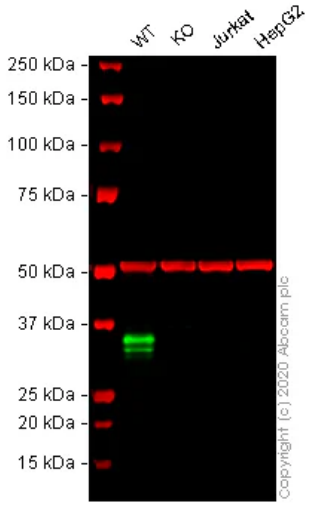
CD74 Flow Cytometry (Intracellular) staining using mouse Anti-CD74 antibody

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 can also be used in Raji cells fixed with 4% formaldehyde (10 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Western blot - Anti-CD74 antibody [LN2] (ab9514)

CD74 Western blot staining using mouse Anti-CD74 antibody

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.

All lanes:
Western blot - Anti-CD74 antibody [LN2] (ab9514) at 5 µg/mL

Lane 1:
Wild-type Raji cell lysate at 30 µg

Lane 2:
Western blot - Human CD74 knockout Raji cell lysate (ab275529) at 30 µg

Lane 3:
Jurkat cell lysate at 30 µg

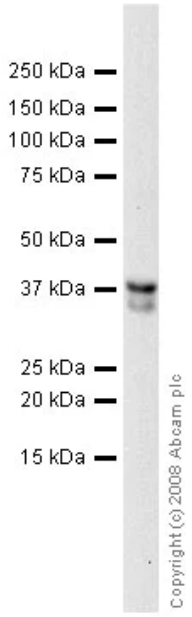
Lane 4:
HepG2 cell lysate at 30 µg

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 35 kDa

Western blot - Anti-CD74 antibody [LN2] (ab9514)



All lanes:
Western blot - Anti-CD74 antibody [LN2] (ab9514) at 5 µg/mL

All lanes:
Human tonsil normal tissue lysate - total protein (ab29615) at 10 µg

Secondary

All lanes:
Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa, 37 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.