abcam

Product datasheet

Anti-CD74 antibody [LN2] ab9514

KO VALIDATED

2 Abreviews 22 References 7 Images

Overview

Product name	Anti-CD74 antibody [LN2]
Description	Mouse monoclonal [LN2] to CD74
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to Human CD74. SU-DHL-4 lymphoma cells
Positive control	Human tonsil normal tissue lysate - total protein: Hum an tonsil and Human liver tissues; Raji cells
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). Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.3 Preservative: 0.1% Sodium azide Constituent: 1% BSA
Purity	Protein A/G purified
Clonality	Monoclonal
Clone number	LN2
Myeloma	unknown
Isotype	IgG1

Light chain type kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab9514 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
Flow Cyt		1/10. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB		Use a concentration of 5 µg/ml. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IHC-P	★★★★★ (2)	1/25 - 1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use a concentration of 1 µg/ml.

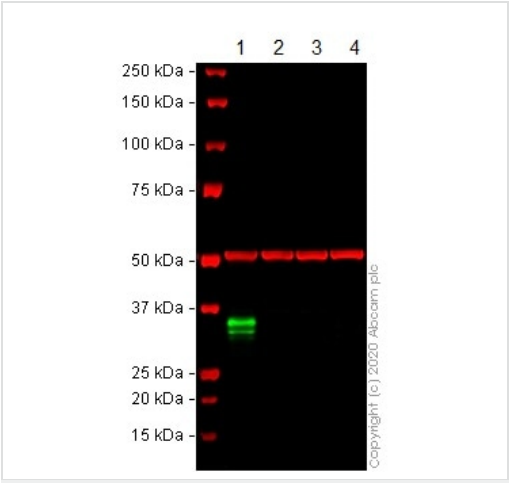
Target

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 [LN2] (ab9514)

All lanes : Anti-CD74 antibody [LN2] (ab9514) at 5 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

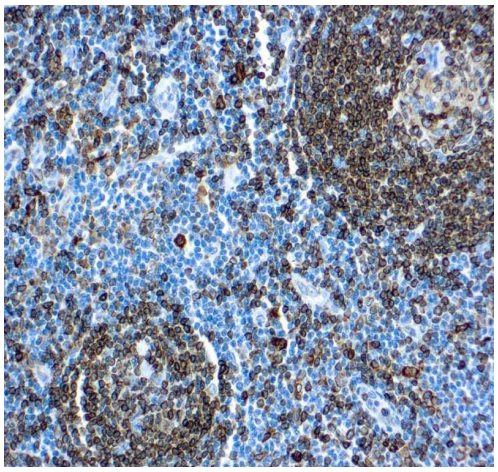
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

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

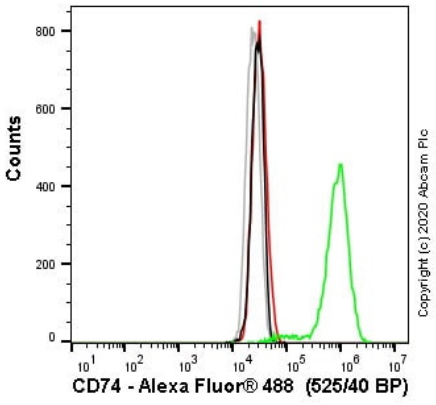
Lanes 1 - 4: Merged signal (red and green). Green - ab9514 observed at 35 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab9514 was shown to react with CD74 in western blot. The band observed in CD74 CRISPR/Cas9 edited cell line **ab273378** (CRISPR/Cas9 edited cell 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.



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

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)

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).

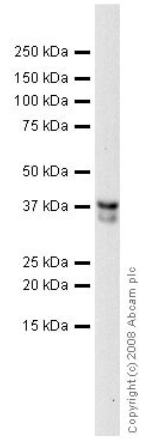
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

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.

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

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 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. 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.

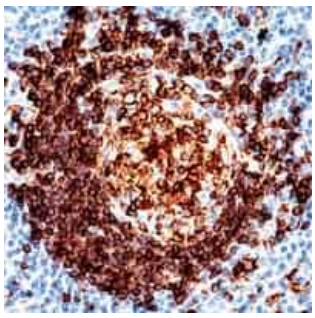


Western blot - Anti-CD74 antibody [LN2] (ab9514)

Anti-CD74 antibody [LN2] (ab9514) at 5 µg/ml + Human tonsil normal tissue lysate - total protein (**ab29615**) at 10 µg

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

Predicted band size: 34 kDa
Observed band size: 34 kDa
Additional bands at: 37 kDa (possible post-translational modification)



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

Ab9514 staining CD74 in paraffin embedded Human tonsil tissue sections by Immunohistochemistry (IHC-P).

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

Image courtesy of an anonymous Abreview.

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.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors