abcam

Product datasheet

Anti-CD74 antibody [EPR4064] ab108393

敲除验证 重组 RabMAb

17 图像

概述

产品名称	Anti-CD74抗体[EPR4064]
描述	兔单克隆抗体[EPR4064] to CD74
宿主	Rabbit
经测试应用	**适用于:** Indirect ELISA, WB, IHC-P, ICC/IF, Flow Cyt (Intra), IHC-Fr **不适用于:** IP
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	Daudi, Jurkat, Raji and Ramos cell lysate; Human tonsil tissue; Raji cells. IHC-Fr: Frozen human tonsil tissue sections
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
存储溶液	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR4064
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab108393于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

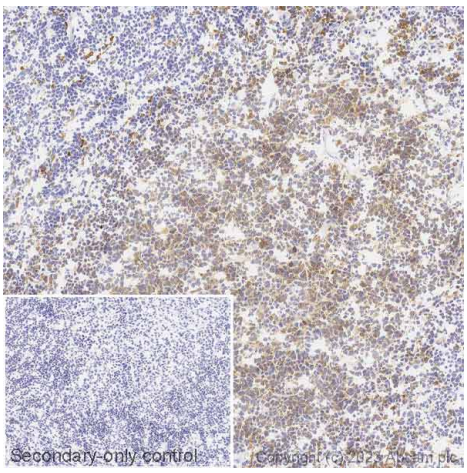
应用	Ab评论	说明
Indirect ELISA		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Predicted molecular weight: 34 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Perform antigen retrieval before commencing with IHC staining protocol.
ICC/IF		1/500.
Flow Cyt (Intra)		Use a concentration of 0.2 µg/ml.
IHC-Fr		Use a concentration of 0.1 µg/ml.

应用说明 Is unsuitable for IP.

靶标

功能	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.
序列相似性	Contains 1 thyroglobulin type-1 domain.
细胞定位	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.

图片

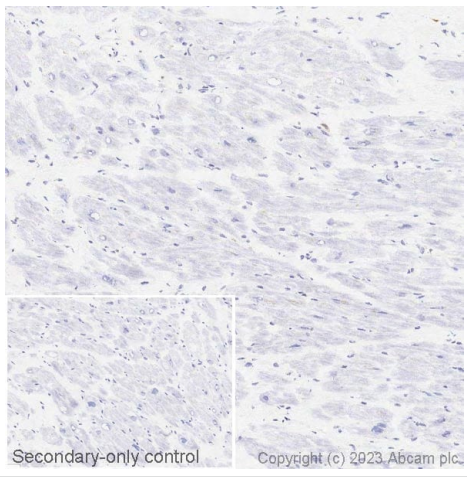


Immunohistochemistry (Frozen sections) - Anti-CD74 antibody [EPR4064] (ab108393)

IHC image of CD74 staining in a section of frozen human normal tonsil* performed on a Leica Biosystems BOND® RX instrument using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108393, 0.05ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Frozen sections) - Anti-CD74 antibody [EPR4064] (ab108393)

Negative control image: IHC image of CD74 staining in a section of frozen human normal heart* performed on a Leica Biosystems BOND® RX instrument using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab108393, 0.05ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

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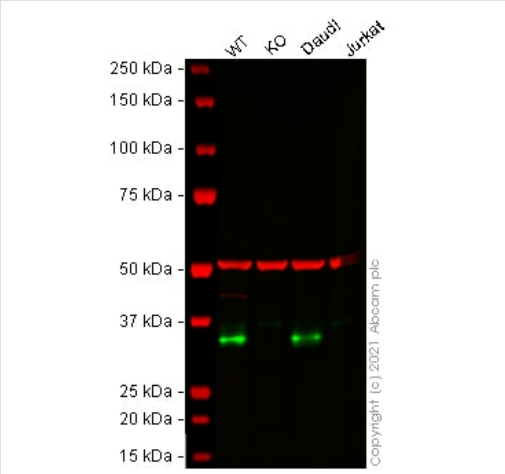


Western blot - Anti-CD74 antibody [EPR4064] (ab108393)

All lanes : Anti-CD74 antibody [EPR4064] (ab108393) at 1/1000 dilution

Lane 1 : Wild-type Raji cell lysate
Lane 2 : CD74 knockout Raji cell lysate
Lane 3 : Daudi cell lysate
Lane 4 : Jurkat cell lysate

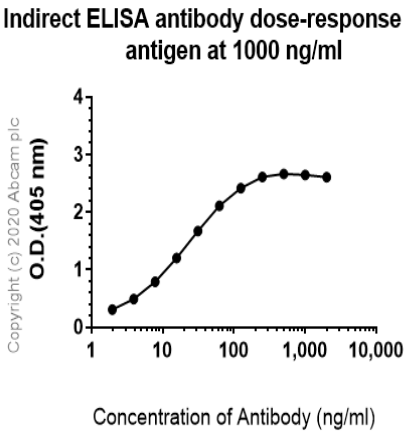
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

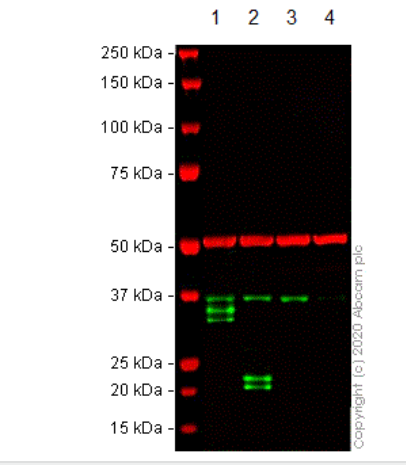
Lanes 1 - 4: Merged signal (red and green). Green - ab108393 observed at 34 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab108393 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 fluorescent western blot (TBS-based) blocking solution before incubation with ab108393 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution 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.



Indirect ELISA - Anti-CD74 antibody [EPR4064] (ab108393)

ELISA using ab108393 at varying antibody concentrations and antigen concentration at 1000 ng/mL. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) was used as the secondary antibody.



Western blot - Anti-CD74 antibody [EPR4064] (ab108393)

All lanes : Anti-CD74 antibody [EPR4064] (ab108393) at 1/1000 dilution

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.

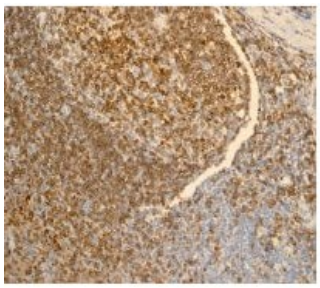
Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab108393 observed at 35 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab108393 was shown to react with CD74 in western blot. The band observed in CD74 CRISPR/Cas9 edited cell line **ab273378**

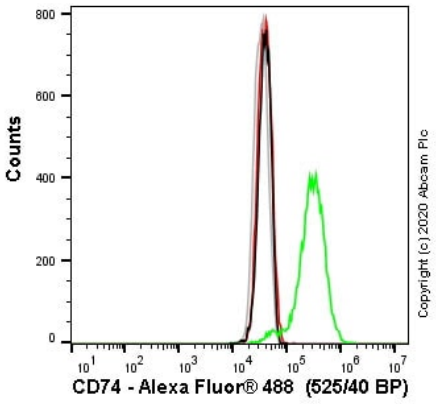
(CRISPR/Cas9 edited lysate **ab275529**) below 35 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab108393 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution 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 [EPR4064] (ab108393)

Immunohistochemical analysis of CD74 in paraffin embedded Human tonsil tissue, using ab108393 at a 1/100 dilution.

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

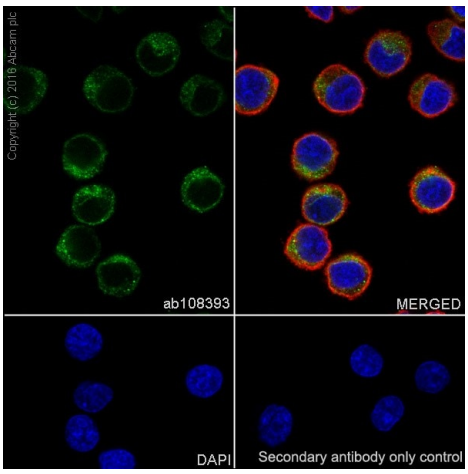


Flow Cytometry (Intracellular) - Anti-CD74 antibody [EPR4064] (ab108393)

Intracellular Intracellular Flow Cytometry overlay histogram showing wild-type Raji (green line) and CD74 knockout Raji cells (**ab273378**) stained with ab108393 (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 (ab108393) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluorr® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 22°C. Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) 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).

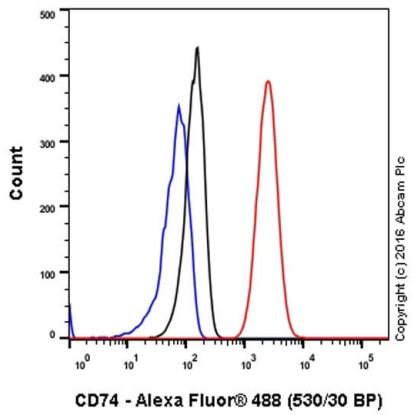
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-CD74 antibody [EPR4064] (ab108393)

Confocal image of ab108393 staining CD74 in the RAMOS (human Burkitt's lymphoma) cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500) and an Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (**ab150077**) was used as the secondary antibody at a dilution of 1/1000.

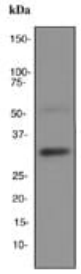
DAPI was used ad a nuclear counterstain at 1/200.



Flow Cytometry (Intracellular) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393staining CD74 inRAMOS (human Burkitt's lymphoma) cell line.The sample was fixed with 4% paraformaldehyde and incubated with the primary antibody (1/150) for 30 minutes at 4°C. AnAlexa Fluorr® 488 -conjugated goatanti-rabbit IgG (1/2000) was used as the secondary antibody.

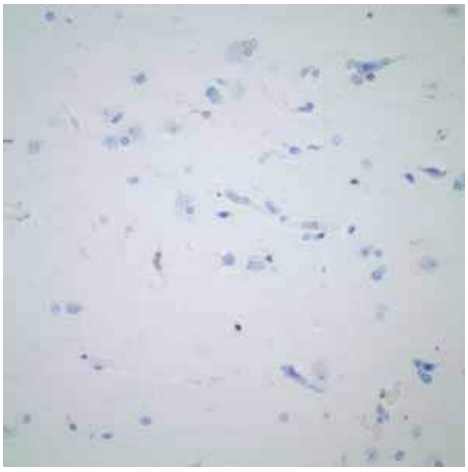
Control:Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-CD74 antibody [EPR4064] (ab108393)

Anti-CD74 antibody [EPR4064] (ab108393) at 1/1000 dilution + Ramos cell lysate at 10 µg

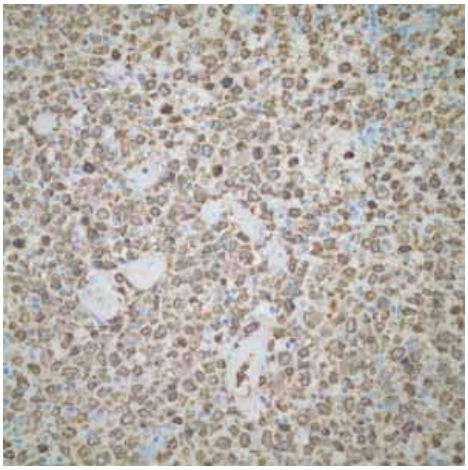
Predicted band size: 34 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing negative staining in Normal brain tissue.

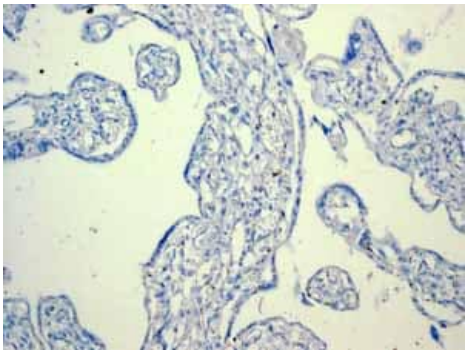
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing positive staining in B cell lymphoma tissue.

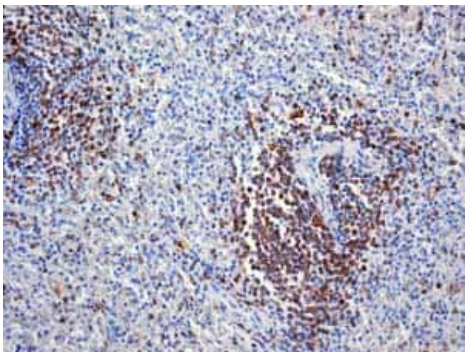
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing negative staining in Normal placenta tissue.

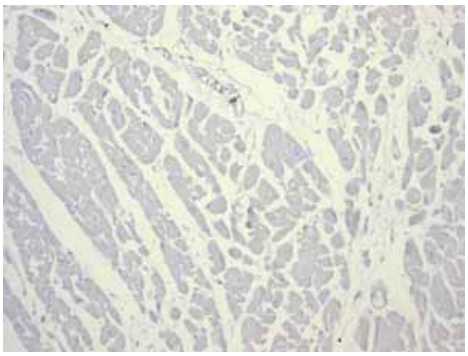
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing positive staining in Normal spleen tissue.

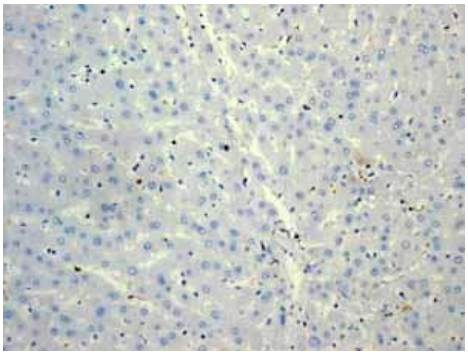
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing negative staining in Normal heart tissue.

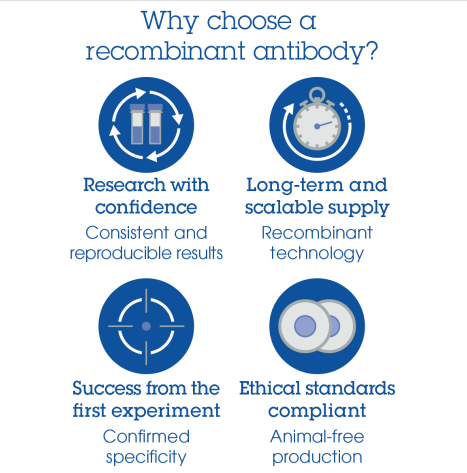
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD74 antibody [EPR4064] (ab108393)

ab108393 showing negative staining in Normal liver tissue.

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



Anti-CD74 antibody [EPR4064] (ab108393)

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