Product datasheet

Anti-CD74 antibody ab64772

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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 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 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

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.
**Function**
Play 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**

**Images**
- 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% H₂O₂/ 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.
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.


Band: 34kDa; CD74

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

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% H2O2 / 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
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 Haematoxylin.

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.

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

This image is courtesy of an abreview submitted by Antibody Solutions Ltd.

ICC/IF image of ab64772 stained RAW264.7 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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