Product datasheet

Anti-CD45 antibody ab10558

Overview

Product name: Anti-CD45 antibody
Description: Rabbit polyclonal to CD45
Host species: Rabbit
Tested applications: Suitable for: Flow Cyt, IHC-Fr, WB, IHC-P, ICC/IF
Species reactivity: Reacts with: Mouse, Rat, Human, Pig, Rhesus monkey
Immunogen: Synthetic peptide corresponding to Human CD45 aa 900-1000 conjugated to Keyhole Limpet Haemocyanin (KLH).
(Peptide available as ab17553, ab17553, ab17553, ab17553)
Positive control: This antibody gave a positive signal in Jurkat and Raw264.7 whole cell lysates, and Rat and Mouse spleen tissue lysates. In IHC this antibody gave a positive signal in Human spleen tissue sections. In Flow Cytometry, this antibody gave a positive signal in methanol fixed Jurkat cells.
General notes: Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).
See other anti-rabbit secondary antibodies that can be used with this antibody.

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
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 ab10558 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
Protein tyrosine-protein phosphatase required for T-cell activation through the antigen receptor. Acts as a positive regulator of T-cell coactivation upon binding to DPP4. The first PTPase domain has enzymatic activity, while the second one seems to affect the substrate specificity of the first one. Upon T-cell activation, recruits and dephosphorylates SKAP1 and FYN.

Involvement in disease
Defects in PTPRC are a cause of severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-positive (T(-)B(+)NK(+) SCID) [MIM:608971]. A form of severe combined immunodeficiency (SCID), a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. Patients present in infancy recurrent, persistent infections by opportunistic organisms. The common characteristic of all types of SCID is absence of T-cell-mediated cellular immunity due to a defect in T-cell development. Genetic variations in PTPRC are involved in multiple sclerosis susceptibility (MS) [MIM:126200]. MS is a neurodegenerative disorder characterized by the gradual accumulation of focal plaques of demyelination particularly in the periventricular areas of the brain. Peripheral nerves are not affected. Onset usually in third or fourth decade with intermittent progression over an extended period. The cause is still uncertain.

Sequence similarities

Domain
The first PTPase domain interacts with SKAP1.

Post-translational modifications
Heavily N- and O-glycosylated.

Cellular localization

Application | Abreviews | Notes
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Flow Cyt | | Use 1µg for 10^6 cells. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr | | 1/150.
WB | | 1/500. Detects a band of approximately 190 kDa (predicted molecular weight: 147 kDa). Can be blocked with Human CD45 peptide (ab17553).
IHC-P | | Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF | | Use a concentration of 0.1 - 1 µg/ml.

Target
Function
Protein tyrosine-protein phosphatase required for T-cell activation through the antigen receptor. Acts as a positive regulator of T-cell coactivation upon binding to DPP4. The first PTPase domain has enzymatic activity, while the second one seems to affect the substrate specificity of the first one. Upon T-cell activation, recruits and dephosphorylates SKAP1 and FYN.

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Cellular localization
ab10558 staining CD45 in Human tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Negative control is shown in panel. Blocking was with horse serum (1/75) for 1 hour at room temperature. Samples were incubated with primary antibody (1/10) overnight at 4°C. A Biotin-conjugated Horse anti-mouse polyclonal (1/200) was used as the secondary antibody.

ab10558 staining CD45 in Mouse abdominal wall tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 5% serum for 24 hours at 4°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/100 in milk) for 24 hours at 4°C. A Biotin-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

IHC image of CD45 staining in human spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab10558, 1µg/ml, 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.
Immunohistochemistry (Frozen sections) - Anti-CD45 antibody (ab10558)
Image courtesy of Mr Sam Lee by Abreview.

ab10558 staining CD45 in fetal mouse liver tissue sections 12.5 dpc by Immunohistochemistry (frozen sections). Tissue was fixed with paraformaldehyde and a permeabilization step was performed using 0.1% Tween-20. Samples were then blocked with 5% BSA for 1.5 hours at 20°C followed by incubation with the primary antibody at a 1/150 dilution, for 16 hours at 4°C. A Goat anti-rabbit Alexa Fluor® 594 polyclonal was used as the secondary antibody at a 1/1500 dilution.

Western blot - Anti-CD45 antibody (ab10558)

All lanes: Anti-CD45 antibody (ab10558) at 1 µg/ml

Lane 1: Jurkat (Human) Whole Cell Lysate
Lane 2: RAW 264.7 (Mouse leukaemic monocyte macrophage cell line) Whole Cell Lysate
Lane 3: Spleen (Mouse) Tissue Lysate
Lane 4: Spleen (Rat) Tissue Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 147 kDa
Observed band size: 190 kDa

why is the actual band size different from the predicted?
Additional bands at: 230 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute

This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being
transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab10558 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

CD45 contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.

**Western blot - Anti-CD45 antibody (ab10558)**

**All lanes**: Anti-CD45 antibody (ab10558) at 1/500 dilution

**Lane 1**: Jurkat Whole Cell Lysate

**Lane 2**: Jurkat Whole Cell Lysate with Human CD45 peptide (ab17553)

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

**Predicted band size**: 147 kDa

**Exposure time**: 3 minutes

**Secondary antibody - goat anti-rabbit H&L (HRP) (ab6721)**

Asynchronous KM-H2 cells were pelleted and labeled by indirect immunofluorescence. Cells were stained with ab10558 (1/200) for 30min at 4°C, washed and then stained with goat anti-rabbit alexafluor 488 (1/200). Forward/Side scatter were used to eliminate cellular debris. The accompanying marker was applied such that only 2% of the IgG control was positive. Based on the accompanying image, approximately 8.4% of cells exhibited positive staining for anti-CD45. Since KM-H2 are known to have low levels of CD45 transcripts they are expected to have low levels of CD45, which is reflected in the ~8%. This image is from an Abreview.
ab10558 (1:40) staining CD45 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 B cells in the germinal centres and mantle zone of the follicles and scattered cells of the interfollicular areas (paracortical T and B cells). There is a mild to moderate degree of cytoplasmic staining associated with the membrane staining in these specific cells.

Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Extended Retrieval programme. Slides were blocked in 3% H₂O₂ /4 min / 37°C and incubated with ab10558 (1:40 dilution / 1 hour / 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/...}

Immunohistochemical analysis of formaldehyde fixed human cephalic sections. Primary antibody ab10558 to CD45 incubated at a concentration of 1/100 for 4°C for 18 hours. Secondary antibody used was a goat anti-rabbit conjugated to biotin at a 1/200 dilution. Blocking was done with serum at a 10% concentration for 1 hour at 25°C.

Overlay histogram showing Jurkat cells stained with ab10558 (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 nonspecific protein-protein interactions. The cells were then incubated with the antibody (ab10558, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/1000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (1µg/1x10⁶ 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.
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