abcam

ab214437

Anti-CD45 antibody [EP322Y] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP322Y
Purification technique	Affinity purification Protein A
Specificity	This antibody recognizes cytoplasmic domain of CD45.
Dissociation constant	3.6×10^{-11} M
Concentration	1.04 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human

Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information PTPRC

Function Protein tyrosine-protein phosphatase required for T-cell activation through the antigen receptor (PubMed:35767951). 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. Dephosphorylates LYN, and thereby modulates LYN activity (By similarity). Interacts with CLEC10A at antigen presenting cell-T cell contact; CLEC10A on immature dendritic cells recognizes Tn antigen-carrying PTPRC/CD45 receptor on effector T cells and modulates T cell activation threshold to limit autoreactivity.

(Microbial infection) Acts as a receptor for human cytomegalovirus protein UL11 and mediates binding of UL11 to T-cells, leading to reduced induction of tyrosine phosphorylation of multiple signaling proteins upon T-cell receptor stimulation and impaired T-cell proliferation.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab214437 is the carrier-free version of ab40763.

Species reactivity

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

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

Activity summary	CD45 also known as Protein tyrosine phosphatase receptor type C (PTPRC) is a transmembrane glycoprotein with a molecular mass ranging between 180-240 kDa depending on its isoform. It is expressed on the surface of almost all hematopoietic cells except for mature erythrocytes and platelets. CD45 has a critical role in regulating antigen receptor signaling by modifying kinases involved in signal transduction and it is essential in lymphocyte development and activation. Because of its broad expression on immune cells CD45 is a valuable marker for differentiating various immune cell types in assays like flow cytometry and immunohistochemistry (IHC) often referred to as CD45 stain.
Biological function summary	CD45 acts by regulating tyrosine phosphorylation in the immune cell signaling context participating directly in signal transduction. It functions by dephosphorylating specific phosphotyrosine residues on various proteins modulating the signaling threshold required for lymphocyte activation. Although not known to be part of a protein complex CD45 itself shows isoform variation that associates with specific immune cell types impacting their function. Importantly CD45 interacts with multiple signaling molecules to affect cell growth and differentiation.
Pathways	Scientists associate CD45 with signaling pathways such as the T-cell receptor (TCR) and B-cell receptor (BCR) signaling. CD45 modulates the activity of Src family kinases important elements in these pathways making it essential for effective immune response and tolerance. Furthermore interactions between CD45 and proteins like Lck in T-cells or Lyn in B-cells highlight its pivotal role in executing its signaling functions.
Associated diseases and disorders	Scientists often link CD45 to immune-related conditions including autoimmunity and leukemia. In autoimmune diseases altered CD45 expression or activity can disrupt normal immune function contributing to pathogenesis. In leukemia CD45 expression levels can assist in disease classification and prognosis as it interacts with proteins involved in cell cycle regulation. The anti-CD45 antibodies can provide diagnostic and therapeutic avenues highlighting their utility in disease management.

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.

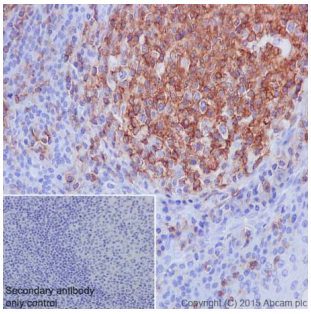
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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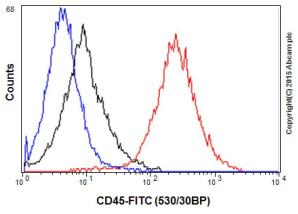
14 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD45 antibody

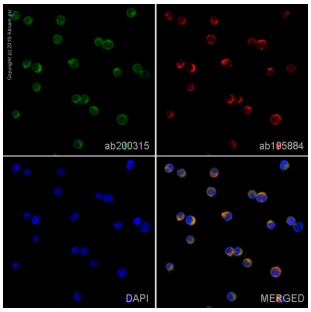
This IHC data was generated using the same anti-CD45 antibody clone, EP322Y, in a different buffer formulation (cat# ab40763).
Immunohistochemical staining of paraffin embedded human tonsil with purified ab40763 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Flow Cytometry (Intracellular) staining using rabbit Anti-CD45 antibody

Overlay histogram showing Jurkat cells fixed in 4% PFA and stained with purified ab40763 at a dilution of 1 in 20 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).



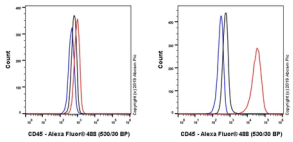
Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD45 antibody

Clone EP322Y (ab214437) has been successfully conjugated by Abcam. This image was generated using Anti-CD45 antibody [EP322Y] (Alexa Fluor® 488). Please refer to ab200315 for protocol details.
ab200315 staining CD45 in Daudi cells. The cells were fixed with 80% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab200315 at 1/250 dilution (shown in green) and ab195884, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labeled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

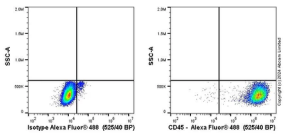
This product also gave a positive signal under the same testing conditions in Daudi cells fixed with 4% formaldehyde (10 min).



Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Flow Cytometry (Intracellular) staining using rabbit Anti-CD45 antibody

This data was developed using ab40763, the same antibody clone in a different buffer formulation.Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 293T (Human embryonic kidney epithelial cell, Left) / Jurkat (Human T cell leukemia T lymphocyte, Right) cells labelling CD45 with ab40763 at 1/500 dilution (0.1ug)/ Right compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. Negative control: 293T. (PMID: 16005866)



Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

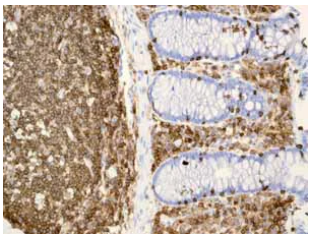
CD45 Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cells using rabbit Anti-CD45 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).
Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab40763 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left).

PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interactions. Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min, followed by the addition of antibody ab40763 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6 in 100 µl at 0.2 µg/ml (1/10,500)) for 30 min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C.

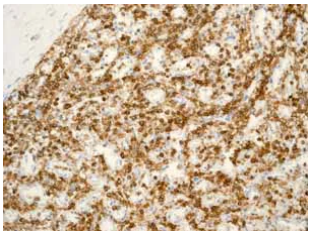
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD45 antibody

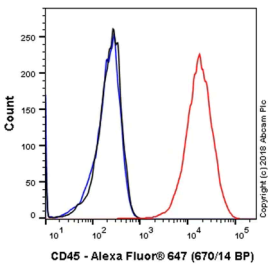
Unpurified ab40763 showing positive staining in Normal colon lymphoid tissue.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD45 antibody

Unpurified ab40763 showing positive staining in Normal spleen tissue.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).



Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

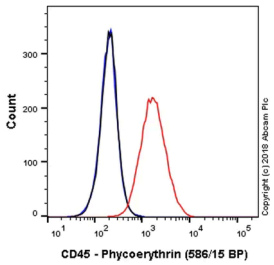
CD45 Flow Cytometry (Intracellular) staining using rabbit Anti-CD45 antibody

Clone EP322Y (ab214437) has been successfully conjugated by Abcam. This image was generated using Anti-CD45 antibody [EP322Y] (Alexa Fluor® 647). Please refer to ab200317 for protocol details.
Overlay histogram showing Jurkat (human T cell leukemia cell line from peripheral blood) cells stained with ab200317 (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 / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab200317, 1/50000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Alexa Fluor® 647 (ab199093) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 40 mW Red laser (640nm) and 670/14 bandpass filter.

This antibody gave a positive signal in Jurkat 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 (Intracellular) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

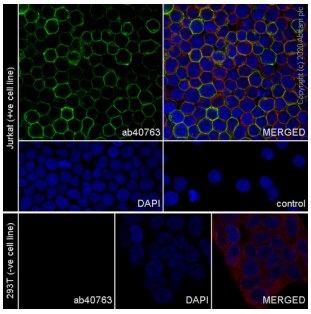
CD45 Flow Cytometry (Intracellular) staining using rabbit Anti-CD45 antibody

Clone EP322Y (ab214437) has been successfully conjugated by Abcam. This image was generated using Anti-CD45 antibody [EP322Y] (PE). Please refer to ab214501 for protocol details.
Overlay histogram showing Jurkat (human T cell leukemia cell line from peripheral blood) cells stained with ab214501 (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 / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab214501, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

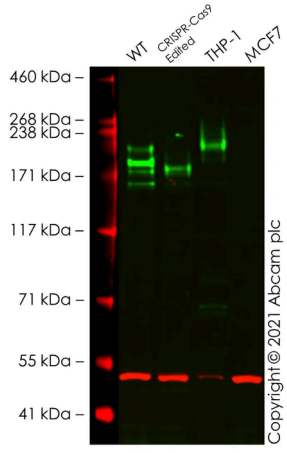
This antibody gave a positive signal in Jurkat cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD45 antibody

This data was developed using ab40763, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Jurkat cells labelling CD45 with ab40763 at 1/100 (6.1 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing membranouse staining in Jurkat cells and no staining in 293T cells. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 2ug/ml dilution.



Western blot - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

CD45 Western blot staining using rabbit Anti-CD45 antibody

False colour image of Western blot: Anti-CD45 antibody [EP322Y] staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40763 was shown to bind specifically to CD45. A band was observed at 160-220 kDa in wild-type Jurkat cell lysates with no signal observed at this size in PTPRC CRISPR-Cas9 edited cell line ab274932 (CRISPR-Cas9 edited cell lysate ab274990). The band observed in the CRISPR-Cas9 edited lysate lane below 160-220 kDa is likely to represent a truncated form of CD45. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and PTPRC CRISPR-Cas9 edited Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 %

Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution

Lane 1:
Wild-type Jurkat cell lysate at 20 µg

Lane 2:
PTPRC CRISPR-Cas9 edited Jurkat cell lysate at 20 µg

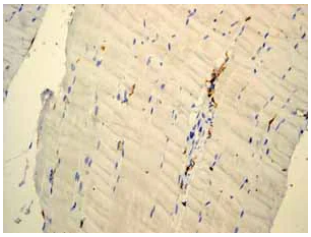
Lane 3:
THP-1 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Performed under reducing conditions.

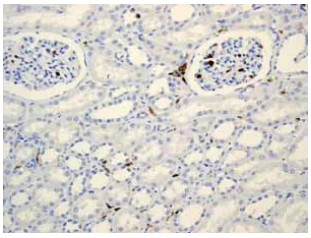
Predicted band size: 147 kDa

Observed band size: 160-220 kDa



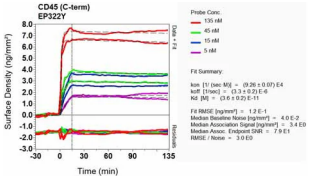
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

Unpurified ab40763 showing negative staining in Skeletal muscle tissue.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

Unpurified ab40763 showing negative staining in Normal kidney tissue.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).



OI-RD Scanning - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40763).

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