abcam

ab40763

Anti-CD45 antibody [EP322Y]

Anti-CD45 antibody [EP322Y] (ab40763) is a rabbit monoclonal antibody detecting CD45 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 50 publications
- Trusted since 2006

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
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	0.073 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/5000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/15 - 1/20
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. Permeabilization and intracellular staining is necessary.

IHC-P

Tested

Species	Human
Dilution info	1/250
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-CD45 antibody [EP322Y] (ab40763) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P and WB.

Abcam's high quality manufacturing and validation processes ensure Anti-CD45 antibody [EP322Y] (ab40763) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-CD45 antibody [EP322Y] (ab40763) has 13 independent reviews from customers.

Anti-CD45 antibody [EP322Y] (ab40763) specifically detects CD45 (UniProt ID: P08575; Molecular weight: 145kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EP322Y - ab214437.

Antibody clone EP322Y is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, PE, PerCP, APC, Alexa Fluor® 594 (ab200315, ab200317, ab214501, ab220298, ab221245, ab303598).

CD45 regulates immune cell signaling and is crucial in the development and function of immune cells, with alterations linked to various immunodeficiencies and autoimmune diseases.

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.

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

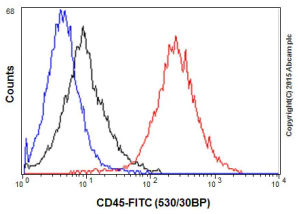
We do not recommend this combination. It is not covered by our product promise.

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.

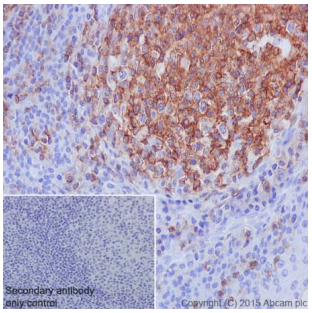
15 product images



Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] (ab40763)

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

Overlay histogram showing Jurkat (Human T cell leukemia cell line from peripheral blood) 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).

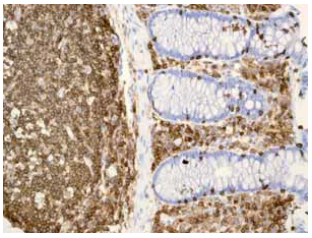


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

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

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 counterstained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

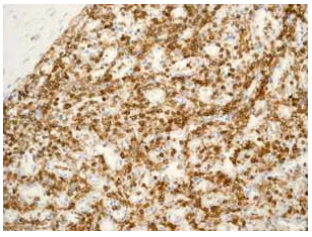
PBS was used instead of the primary antibody as the negative control (shown in the inset).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

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

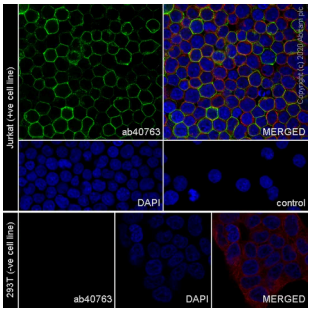
Unpurified ab40763 showing positive staining in normal colon lymphoid cells tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

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

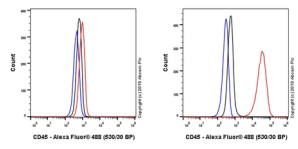
Unpurified ab40763 showing positive staining in normal spleen tissue.



Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] (ab40763)

CD45 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD45 antibody

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.

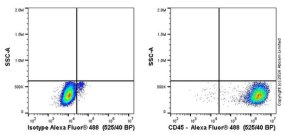


Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] (ab40763)

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

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] (ab40763)

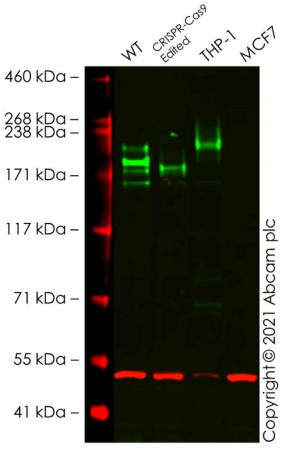
CD45 Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cells using rabbit Anti-CD45 antibody

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.



Western blot - Anti-CD45 antibody [EP322Y] (ab40763)

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



Western blot - Anti-CD45 antibody [EP322Y] (ab40763)

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 3% milk before being incubated with ab40763 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

All lanes:
Western blot - Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution

All lanes:
Jurkat (Human T cell lymphoblast-like cell line) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

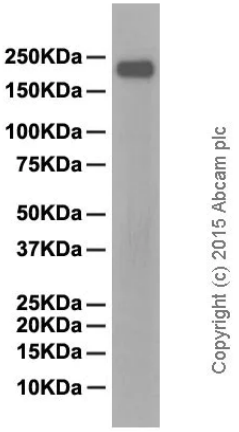
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 147 kDa

Observed band size: 200 kDa

Exposure time: 8min



Western blot - Anti-CD45 antibody [EP322Y] (ab40763)

Blocking/Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution

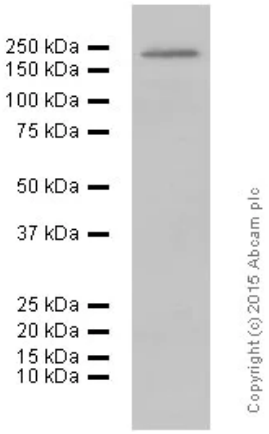
All lanes:
HuT-78 cell lysate at 20 µg

Secondary

All lanes:
HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

Predicted band size: 147 kDa

Observed band size: 200 kDa



Western blot - Anti-CD45 antibody [EP322Y] (ab40763)

CD45 Western blot staining of Raji (Human Burkitt's lymphoma cell line) cell lysate using rabbit Anti-CD45 antibody

Blocking/Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution

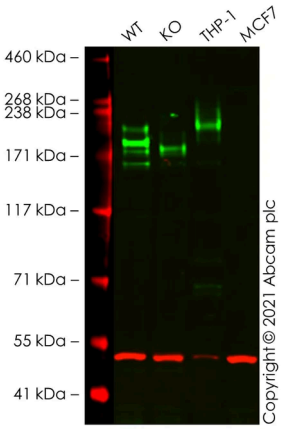
All lanes:
Raji (Human Burkitt's lymphoma cell line) cell lysate at 20 µg

Secondary

All lanes:
HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

Predicted band size: 147 kDa

Observed band size: 200 kDa



Western blot - Anti-CD45 antibody [EP322Y] (ab40763)

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 knockout cell line ab274932 (knockout cell lysate ab274990). The band observed in the knockout 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 knockout 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

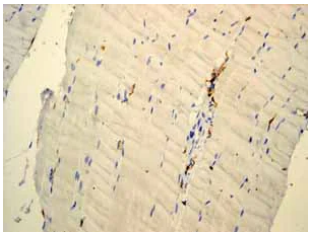
Lane 1:
Wild-type Jurkat cell lysate at 20 µg

Lane 2:
PTPRC knockout Jurkat cell lysate at 20 µg

Lane 3:
THP-1 cell lysate at 20 µg

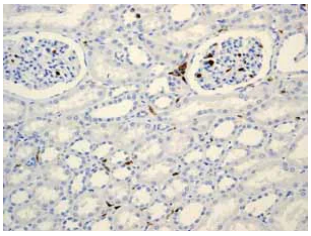
Lane 4:
MCF7 cell lysate at 20 µg

Performed under reducing conditions.



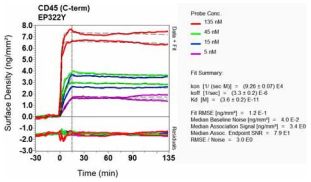
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

Unpurified ab40763 showing **negative staining** in skeletal muscle tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

Unpurified ab40763 showing negative staining in normal kidney tissue.



OI-RD Scanning - Anti-CD45 antibody [EP322Y] (ab40763)

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.

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