abcam

Product datasheet

Anti-CD45 antibody [EP322Y] ab40763

重组 RabMAb

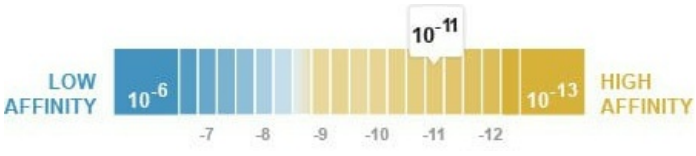
★★★★★ 13 Abreviews 41 References 15 图像

概述

产品名称	Anti-CD45抗体[EP322Y]
描述	兔单克隆抗体[EP322Y] to CD45
宿主	Rabbit
特异性	This antibody recognizes cytoplasmic domain of CD45.
经测试应用	**适用于:** WB, ICC/IF, IHC-P, Flow Cyt (Intra)
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	WB: THP-1, MCF7, Jurkat cell lysate
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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
解离常数 (K_D)	K_D = 3.60 x 10^{-11} M



Learn more about K_D

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EP322Y
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab40763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。

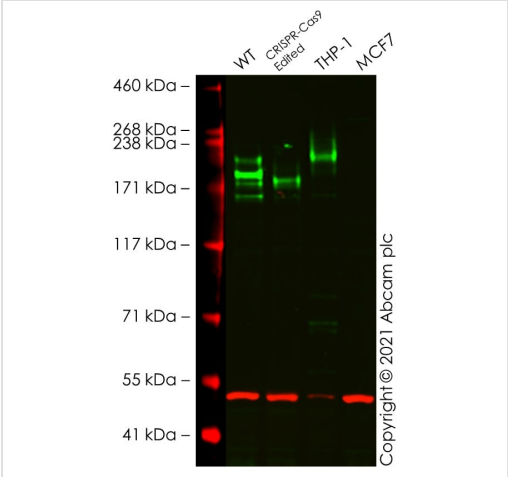
应用	Ab评论	说明
WB	★★★★☆ (1)	1/5000. Detects a band of approximately 200 kDa (predicted molecular weight: 147 kDa).
ICC/IF	★★★★☆ (2)	1/100.
IHC-P	★★★★★ (8)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/15 - 1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. Permeabilization and intracellular staining is necessary.

靶标

功能	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.
疾病相关	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.

序列相似性	Belongs to the protein-tyrosine phosphatase family. Receptor class 1/6 subfamily. Contains 2 fibronectin type-III domains. Contains 2 tyrosine-protein phosphatase domains.
结构域	The first PTPase domain interacts with SKAP1.
翻译后修饰	Heavily N- and O-glycosylated.
细胞定位	Membrane. Membrane raft. Colocalized with DPP4 in membrane rafts.

图片



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

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

Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : PTPRC CRISPR-Cas9 edited Jurkat cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : MCF7 cell lysate

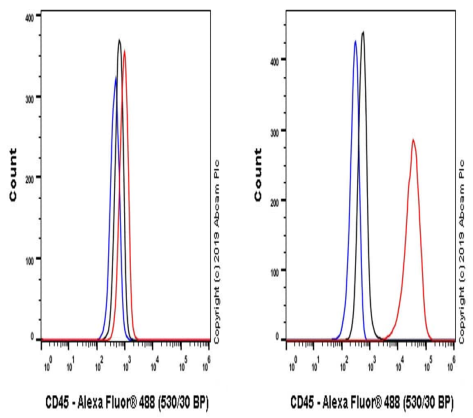
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 147 kDa
Observed band size: 160-220 kDa

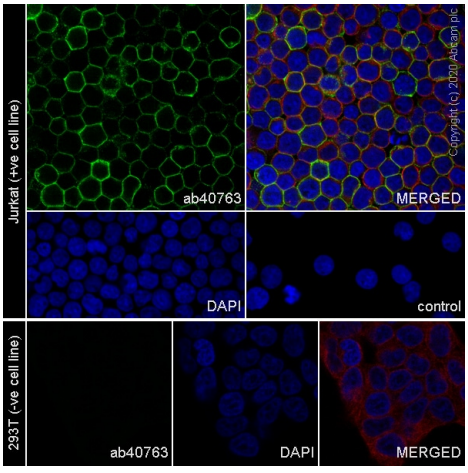
False colour image of Western blot: Anti-CD45 antibody [EP322Y] staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40763 was shown to bind specifically to CD45. A band was observed at 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.



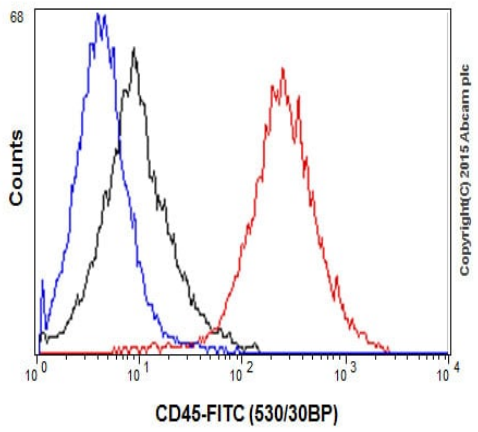
Flow Cytometry (Intracellular) - Anti-CD45 antibody [EP322Y] (ab40763)

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)



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

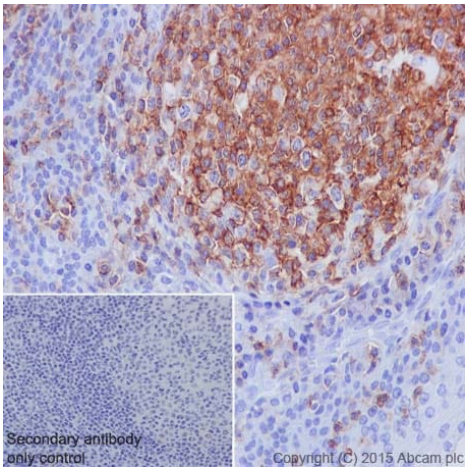
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Jurkat cells labelling CD45 with ab40763 at 1/100 (6.1 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing membranouse 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)

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).

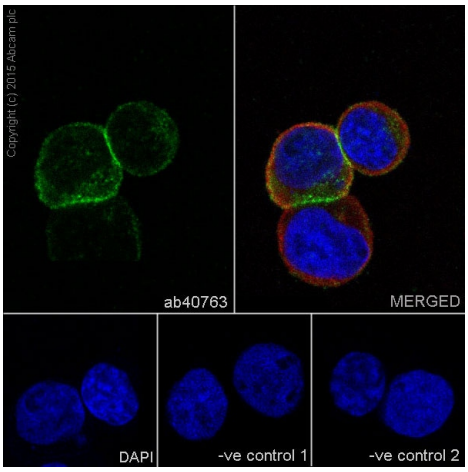


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

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).



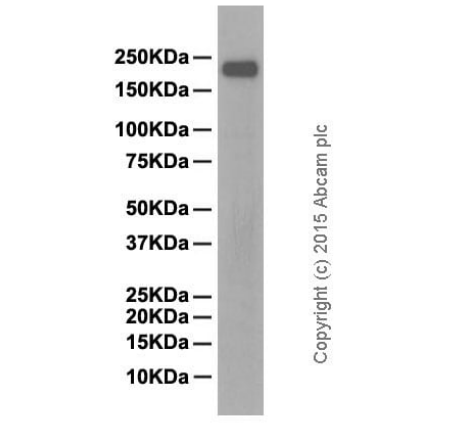
Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] (ab40763)

Immunofluorescence staining of Jurkat cells (Human T cell leukemia cell line from peripheral blood) with purified ab40763 at a working dilution of 1/100, counterstained with DAPI.

The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel.

The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100.

The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab40763 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



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

Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution (purified) + HuT-78 cell lysate at 20 µg

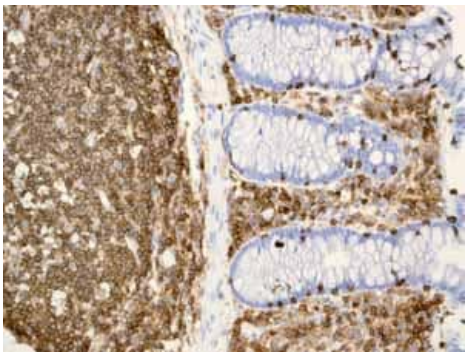
Secondary

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

Predicted band size: 147 kDa

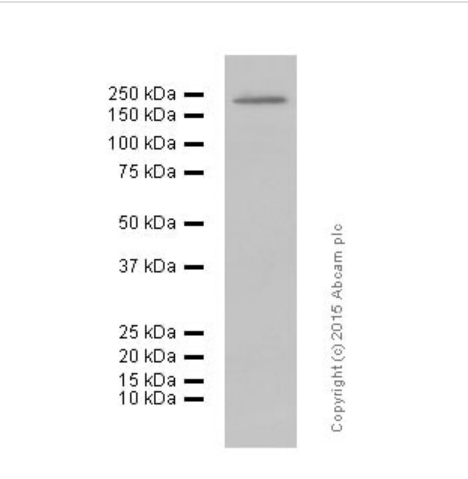
Observed band size: 200 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



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

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



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

Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution (purified) + Raji (Human Burkitt's lymphoma cell line) cell lysate at 20 µg

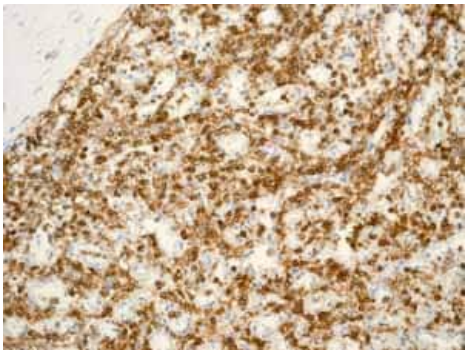
Secondary

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

Predicted band size: 147 kDa

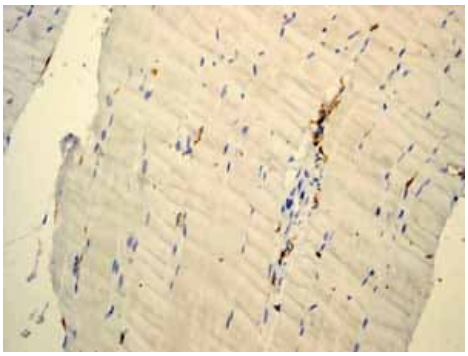
Observed band size: 200 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



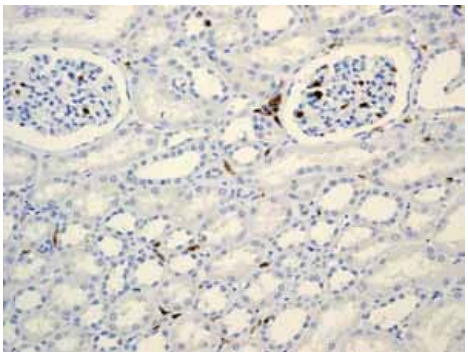
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

Unpurified ab40763 showing positive staining in normal spleen tissue.



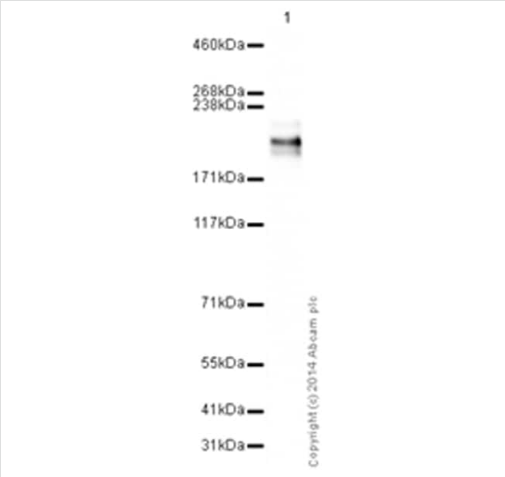
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EP322Y] (ab40763)

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.



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

Anti-CD45 antibody [EP322Y] (ab40763) at 1/5000 dilution (unpurified, blocked with 3% milk) + Jurkat (Human T cell lymphoblast-like cell line) whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Developed using the ECL technique.

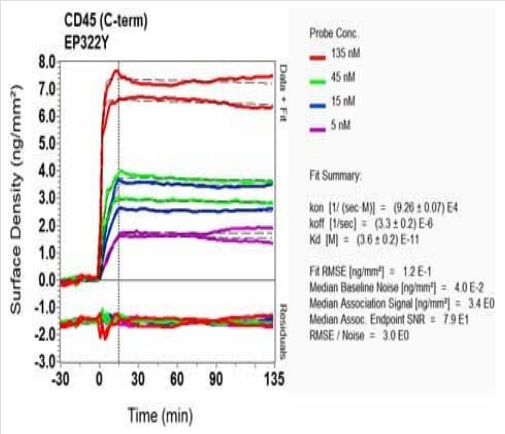
Performed under reducing conditions.

Predicted band size: 147 kDa
Observed band size: 200 kDa

Exposure time: 8 minutes

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**.

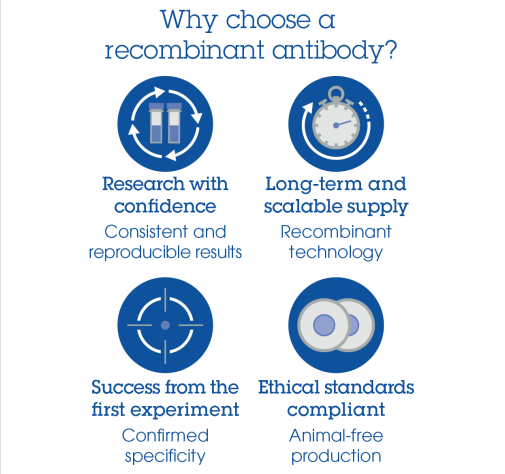


OI-RD Scanning - Anti-CD45 antibody [EP322Y] (ab40763)

Equilibrium disassociation constant (KD)
Learn more about KD

Go here to learn more about KD:

https://www.abcam.com/index.html?pageconfig=resource&rid=15749



Anti-CD45 antibody [EP322Y] (ab40763)

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