abcam

Product datasheet

# Anti-CD45 antibody [EP322Y] - BSA and Azide free ab214437

重组 RabMAb

★★★★★ 1 Abreviews 5 References 15 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-CD45抗体[EP322Y] - BSA and Azide free |
| **描述** | 兔单克隆抗体[EP322Y] to CD45 - BSA and Azide free |
| **宿主** | Rabbit |
| **特异性** | This antibody recognizes cytoplasmic domain of CD45. |
| **经测试应用** | **适用于:** Flow Cyt (Intra), ICC/IF, IHC-P, WB |
| **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: Jurkat and THP-1 cell lysates. Flow Cyt: 293T and Jurkat cells. ICC/IF: Jurkat and Daudi cells. IHC-P: Kidney, colon lymphoid, skeletal muscle, spleen, and Human tonsil tissues. |
| **常规说明** | ab214437 is the carrier-free version of **ab40763**. |

Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

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.

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.

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

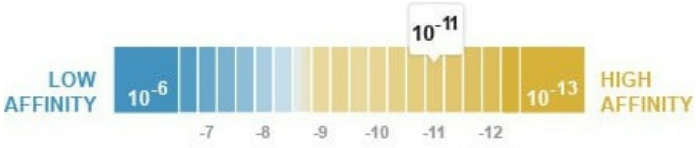
For more information **see here**.

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. Do Not Freeze. |
| **解离常数 ($K_D$)** | $K_D$ = 3.60 x $10^{-11}$ M |




Learn more about $K_D$

| | |
|---|---|
| **存储溶液** | pH: 7.2<br>Constituent: PBS |
| **无载体** | 是 |
| **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ab214437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 |
|---|---|---|
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration.<br>**ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| **ICC/IF** | | Use at an assay dependent concentration. |
| **IHC-P** | ★★★★★ (1) | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 200 kDa (predicted molecular weight: 147 kDa). |

## 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.

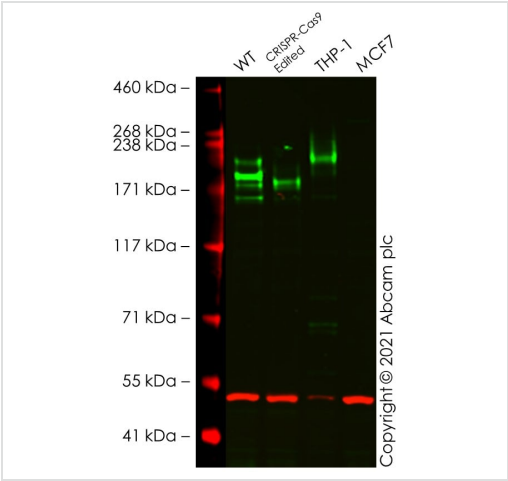
**翻译后修饰**

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] - BSA and Azide free (ab214437)

**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

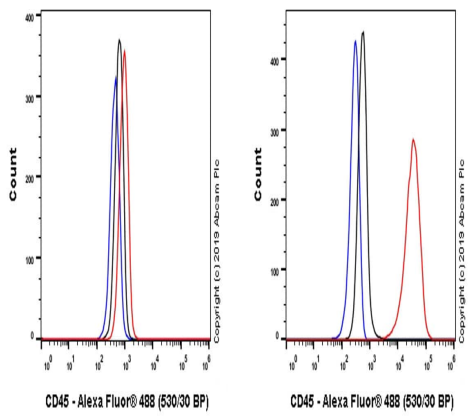
Lysates/proteins at 20 µg per lane.

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-

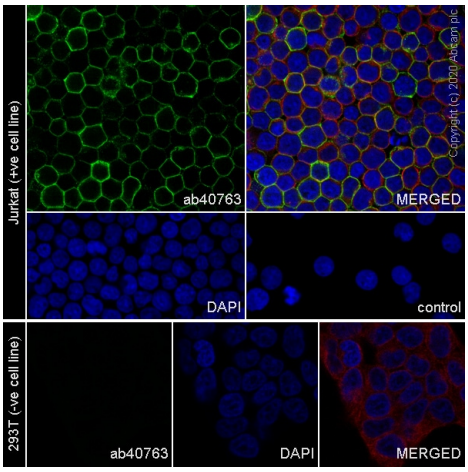
type Jurkat cell lysates with no signal observed at this size in PTPRC CRISPR-Cas9 edited cell line **ab274932** (CRISPR-Cas9 edited cell lysate **ab274990**). The band observed in the CRISPR-Cas9 edited lysate lane below 160-220 kDa is likely to represent a truncated form of CD45. This has not been 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] - BSA and Azide free (ab214437)

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)

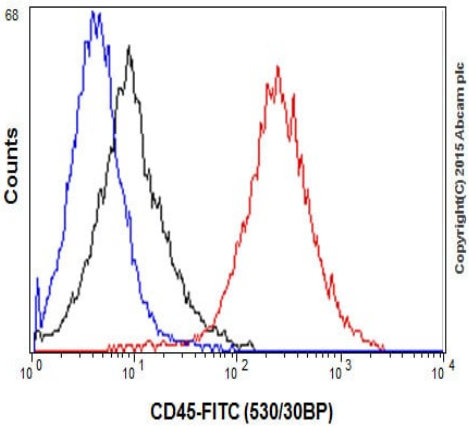



Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

This data was developed using **ab40763**, the same antibody clone in a different buffer formulation.

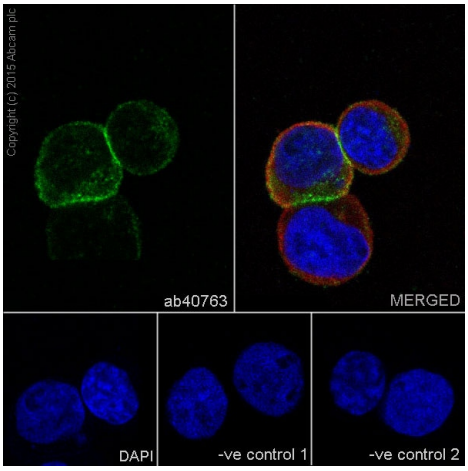
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Jurkat cells labelling CD45 with **ab40763** at 1/100 (6.1 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing membranouse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




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

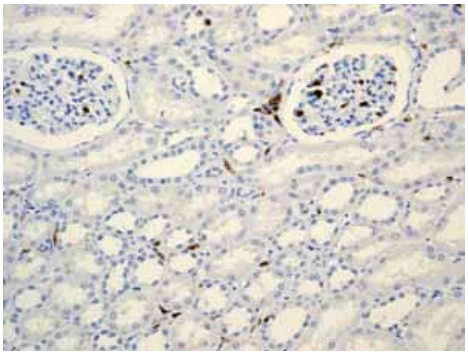
Overlay histogram showing Jurkat cells fixed in 4% PFA and stained with purified **ab40763** at a dilution of 1 in 20 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the 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)

Immunofluorescence staining of Jurkat cells with purified **ab40763** at a working dilution of 1/100, counter-stained 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.

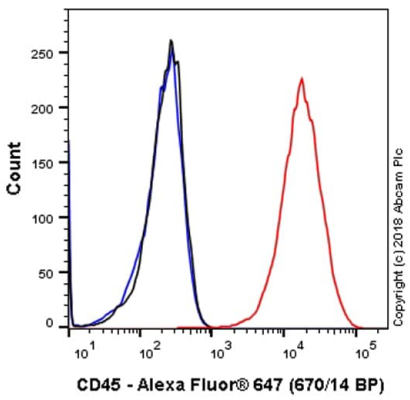
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40763**).



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**).




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

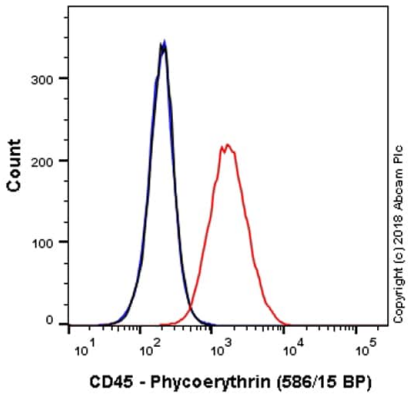
Clone EP322Y (ab214437) has been successfully conjugated by Abcam. This image was generated using Anti-CD45 antibody [EP322Y] (Alexa Fluor® 647). Please refer to **ab200317** for protocol details.

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)

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.

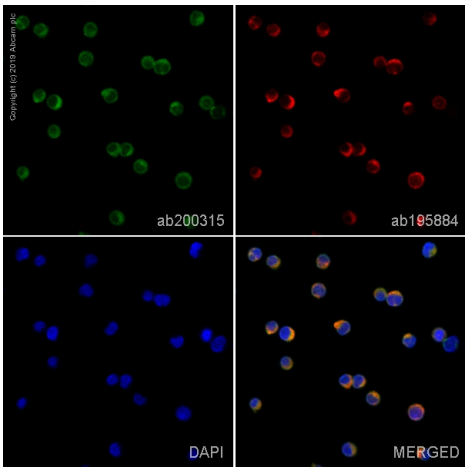
Overlay histogram showing Jurkat (human T cell leukemia cell line from peripheral blood) cells stained with **ab214501** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum 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.



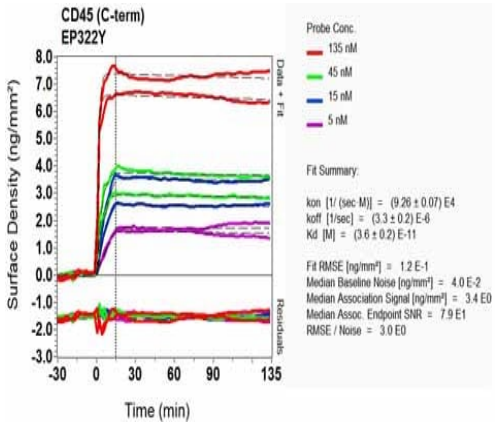

Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

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).



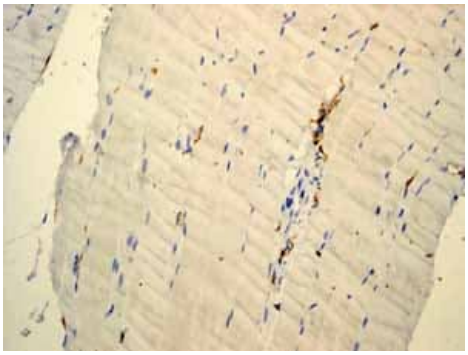

OI-RD Scanning - Anti-CD45 antibody [EP322Y] - BSA and Azide free (ab214437)

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

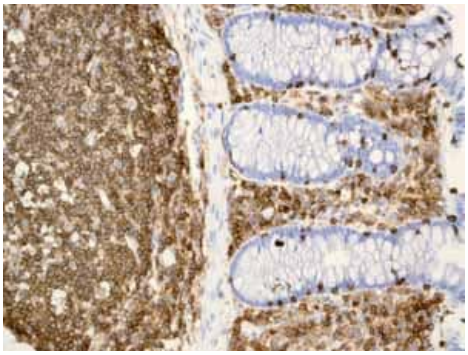
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40763**).



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

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

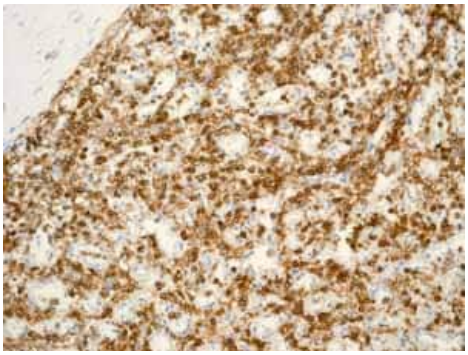
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40763**).



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

Unpurified **ab40763** showing positive staining in Normal colon lymphoid tissue.

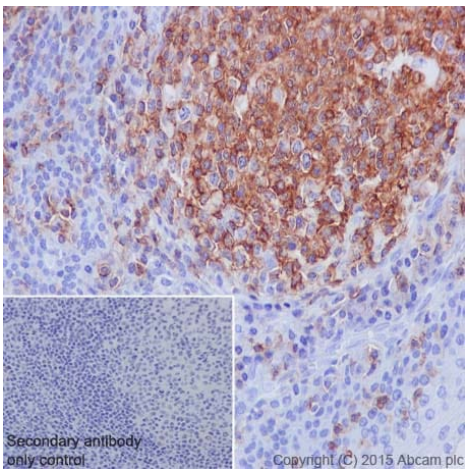
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40763**).



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

Unpurified **ab40763** showing positive staining in Normal spleen tissue.

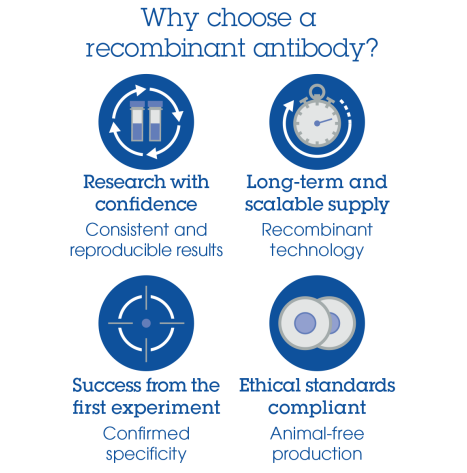
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40763**).




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

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.




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



## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

please visit **https://www.abcam.cn/abpromise** or contact our technical team.

### Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors