abcam

Product datasheet

Anti-CD45 antibody [EPR20033] ab208022

Recombinant RabMAb

★★★★★ 1 Abreviews 7 References 8 Images

Overview

Product name	Anti-CD45 antibody [EPR20033]
Description	Rabbit monoclonal [EPR20033] to CD45
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, IP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: J774A.1, WEHI-231 and Jurkat whole cell lysates; mouse thymus and spleen lysates, THP-1, MCF7. IP: J774A.1 whole cell lysate. IHC-P: Human tonsil tissue and Mouse spleen tissue.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20033
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab208022 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★★ (1)	1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 180, 240 kDa (predicted molecular weight: 145 kDa).
IP		1/30.

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.

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.

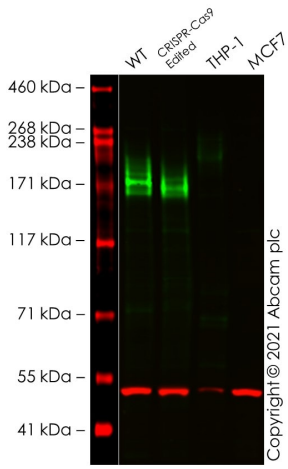
Sequence similarities Belongs to the protein-tyrosine phosphatase family. Receptor class 1/6 subfamily.
Contains 2 fibronectin type-III domains.
Contains 2 tyrosine-protein phosphatase domains.

Domain The first PTPase domain interacts with SKAP1.

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

Cellular localization Membrane. Membrane raft. Colocalized with DPP4 in membrane rafts.

Images



Western blot - Anti-CD45 antibody [EPR20033] (ab208022)

All lanes : Anti-CD45 antibody [EPR20033] (ab208022) at 1/1000 dilution

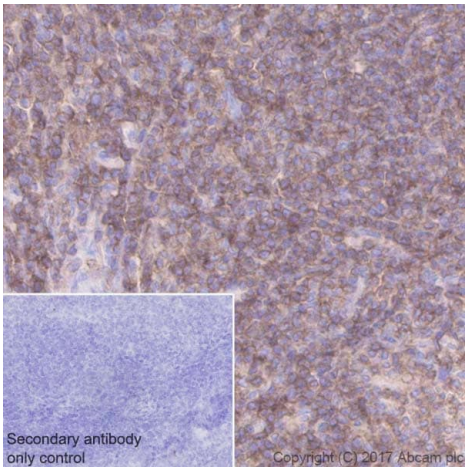
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : PTPRC CRISPR-Cas9 edited Jurkat cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 145 kDa
Observed band size: 160-220 kDa

False colour image of Western blot: Anti-CD45 antibody [EPR20033] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab208022 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.

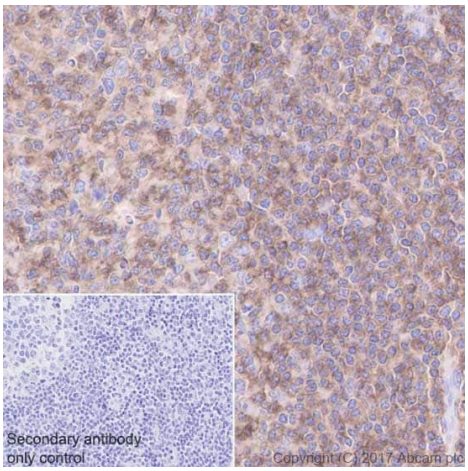


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EPR20033] (ab208022)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue sections labeling CD45 with purified ab208022 at 1/5000 dilution (0.14 µg/ml). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Heat mediated antigen retrieval was performed with Tris/EDTA pH9 buffer (**ab93684**).

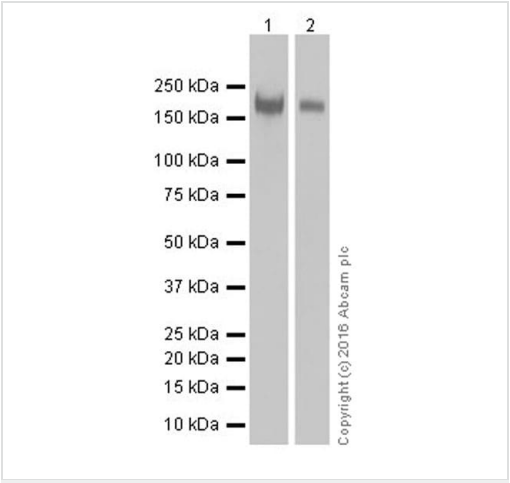


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EPR20033] (ab208022)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling CD45 with purified ab208022 at 1/5000 dilution (0.14 µg/ml). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Heat mediated antigen retrieval was performed with Tris/EDTA pH9 buffer (**ab93684**).



Western blot - Anti-CD45 antibody [EPR20033] (ab208022)

All lanes : Anti-CD45 antibody [EPR20033] (ab208022) at 1/1000 dilution

Lane 1 : J774A.1 (Mouse reticulum cell sarcoma macrophage cell line) whole cell lysate
Lane 2 : WEHI-231 (Mouse B cell lymphoblast cell line) whole cell lysate

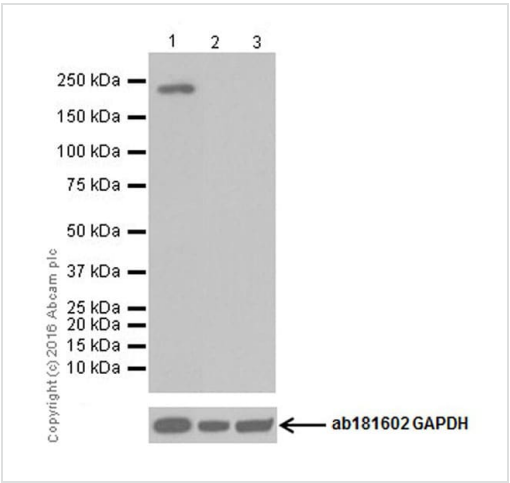
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 145 kDa
Observed band size: 180 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 30 seconds; Lane 2: 5 seconds.



Western blot - Anti-CD45 antibody [EPR20033] (ab208022)

All lanes : Anti-CD45 antibody [EPR20033] (ab208022) at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 2 : 293T (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

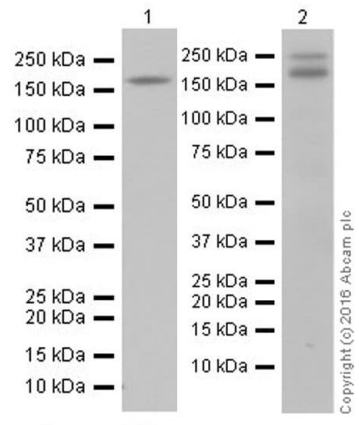
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 145 kDa
Observed band size: 240 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

293T and MCF7 are reported to be CD45 negative cell lines (PMID: 22978632& 24602188).



Western blot - Anti-CD45 antibody [EPR20033] (ab208022)

All lanes : Anti-CD45 antibody [EPR20033] (ab208022) at 1/1000 dilution

Lane 1 : Mouse thymus lysate
Lane 2 : Mouse spleen tissue lysate

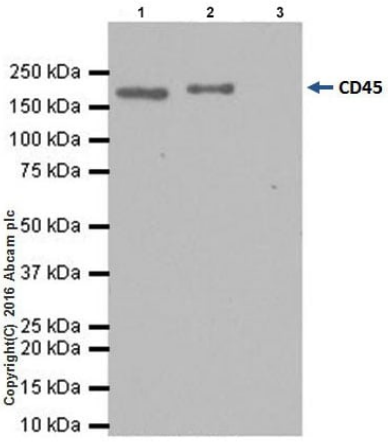
Lysates/proteins at 10 µg per lane.

Secondary
Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution
Lane 2 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 145 kDa
Observed band size: 180,240 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2: 15 seconds.



Immunoprecipitation - Anti-CD45 antibody [EPR20033] (ab208022)

CD45 was immunoprecipitated from 0.35 mg of J774A.1 (Mouse reticulum cell sarcoma macrophage cell line) whole cell lysate with ab208022 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab208022 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

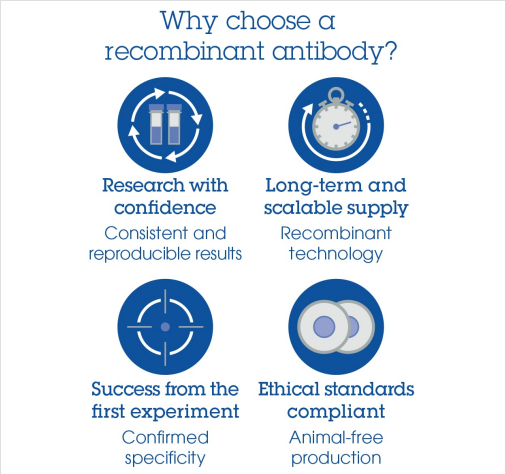
Lane 1: J774A.1 whole cell lysate 10µg (Input).

Lane 2: ab208022 IP in J774A.1 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab208022 in J774A.1 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-CD45 antibody [EPR20033] (ab208022)

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