abcam

Product datasheet

Anti-CD45 antibody [EPR20033] - BSA and Azide free ab229292

Recombinant RabMAb

2 Abreviews 6 Images

Overview

Product name	Anti-CD45 antibody [EPR20033] - BSA and Azide free
Description	Rabbit monoclonal [EPR20033] to CD45 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, WB, IHC-P
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 tissue lysates. IP: J774A.1 whole cell lysate. IHC-P: Human tonsil tissue and Mouse spleen tissue.
General notes	ab229292 is the carrier-free version of **ab208022**. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20033
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab229292 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 180, 240 kDa (predicted molecular weight: 145 kDa).
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.

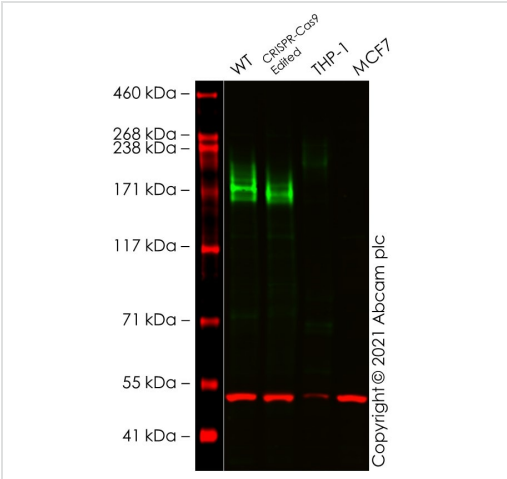
Target

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] - BSA and Azide free (ab229292)

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

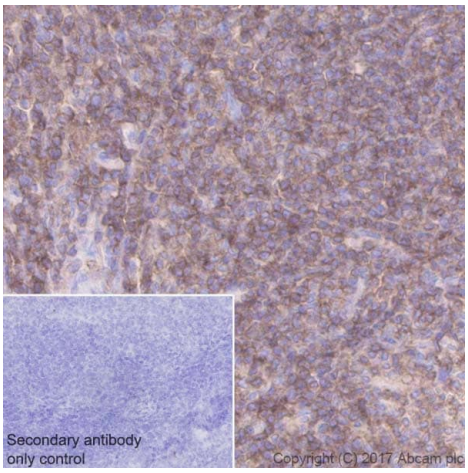
Lysates/proteins at 20 µg per lane.

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.



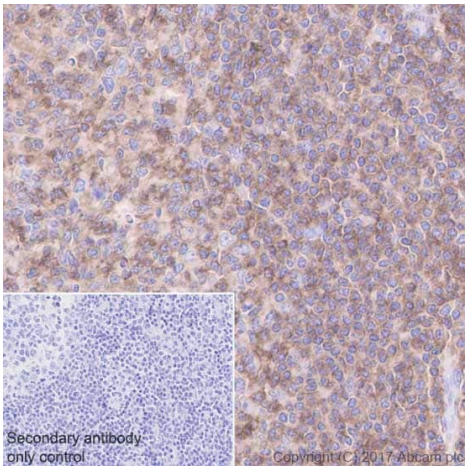
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EPR20033] - BSA and Azide free (ab229292)

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab208022**).



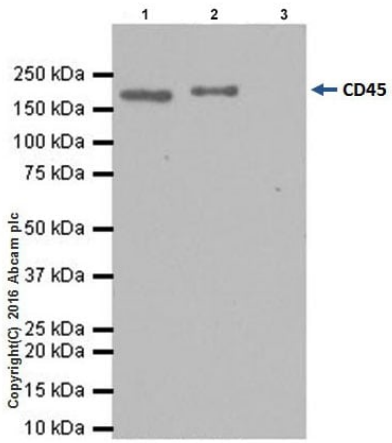
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [EPR20033] - BSA and Azide free (ab229292)

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab208022**).



Immunoprecipitation - Anti-CD45 antibody [EPR20033] - BSA and Azide free (ab229292)

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).

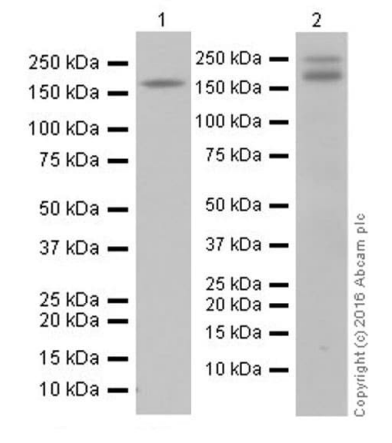
Lane 2: **ab208022** IP in J774A.1 whole cell lysate.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab208022**).



Western blot - Anti-CD45 antibody [EPR20033] - BSA and Azide free (ab229292)

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.

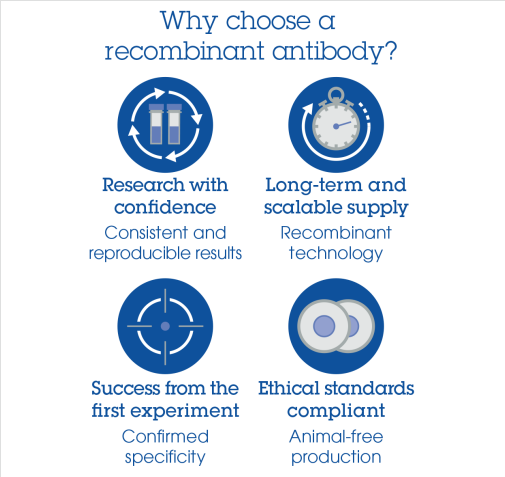
Secondary
Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution
Lane 2 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 145 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab208022**).



Anti-CD45 antibody [EPR20033] - BSA and Azide free (ab229292)

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