# abcam

## Product datasheet

## Anti-CD45 antibody [EPR20033] ab208022



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#### 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **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

1

#### **Applications**

#### The Abpromise quarantee

Our Abpromise quarantee 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.

The first PTPase domain interacts with SKAP1.

Post-translational

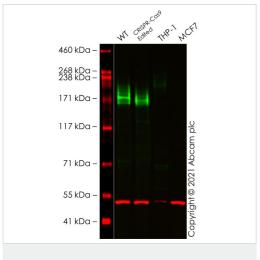
modifications

Heavily N- and O-glycosylated.

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

#### **Images**

**Domain** 



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

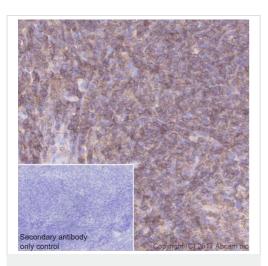
Lysates/proteins at 20 µg per lane.

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<sup>®</sup> 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.

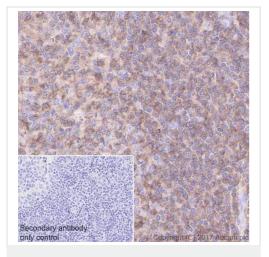


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody
[EPR20033] (ab208022)

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).

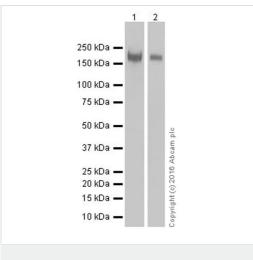


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody
[EPR20033] (ab208022)

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 lgG H&L (HRP) (<u>ab97051</u>) 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.

1 2 3

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

50 kDa —

25 kDa —

910 25 kDa —

910 15 kDa —

910 kDa —

910 kDa —

910 kDa —

4 bd kDa APDH

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 lgG H&L (HRP) (<u>ab97051</u>) 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).

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.



Lane 1: Goat Anti-Rabbit lgG 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.

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).

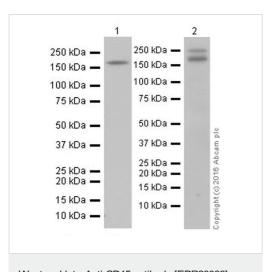
Lane 2: ab208022 IP in J774A.1 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab208022 in

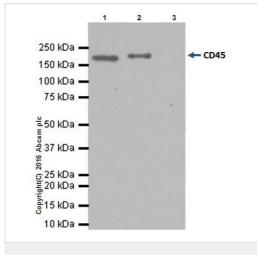
J774A.1 whole cell lysate.

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

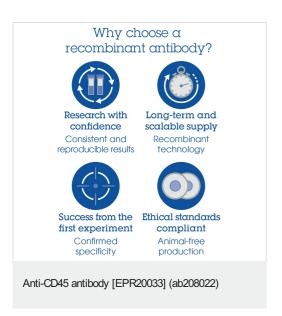
Exposure time: 3 minutes.



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



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



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