abcam

Product datasheet

Anti-CD45 antibody [RM1007] ab281586

Recombinant RabMAb

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Overview

Product name Anti-CD45 antibody [RM1007]

Rabbit recombinant multiclonal [RM1007] to CD45 **Description**

Host species Rabbit

Tested applications Suitable for: IP, Flow Cyt (Intra), WB, IHC-P, ICC

Unsuitable for: IHC-Fr

Species reactivity Reacts with: Mouse. Rat. Human

Immunogen This product was produced with the following immunogens:

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

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Positive control WB: Jurkat, Mouse thymus, Rat thymus lysates. IHC-P: Human tonsil, spleen, colon cancer, Mouse

spleen and Rat spleen tissues. ICC: Jurkat cells. Flow Cyt: Human PBMCs, Jurkat cells, Mouse

PBMCs. IP: Jurkat cell.

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: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity Protein A purified

Clonality Recombinant Multiclonal

Clone number RM1007

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab281586 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		1/50.
Flow Cyt (Intra)		1/50.
WB		1/1000. Predicted molecular weight: 147 kDa.
IHC-P	****(3)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC		1/50.

Application notes

Is unsuitable for IHC-Fr.

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.

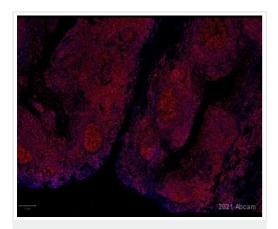
Domain

The first PTPase domain interacts with SKAP1.

Post-translational

Heavily N- and O-glycosylated.

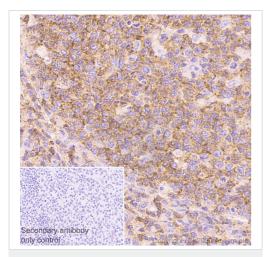
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

This image is courtesy of an Abreview submitted by Dvlan Windell

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of paraformaldehyde-fixed 0.3% Triton X-100 permeabilized human tonsil tissue staining with ab281586 at 10 µg/ml concentration. Samples were incubated with the primary antibody for 1 hour at 20°C. Blocking was done using Donkey Serum 10% + 3% BSA for 24 hours at 4°C. Heat mediated antigen retrieval with Citrate pH 6 & TRIS pH 9.

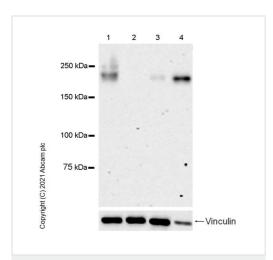


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling CD45 with ab281586 at 1/4000 (0.138 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human tonsil. The section was incubated with ab281586 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-CD45 antibody [RM1007] (ab281586)

All lanes : Anti-CD45 antibody [RM1007] (ab281586) at 1/1000 dilution

Lane 1 : Jurkat (human T cell leukemia T lymphocyte), whole cell lysate

Lane 2: 293T (human embryonic kidney epithelial cell) whole cell lysate

Lane 3 : Mouse thymus tissue lysate

Lane 4 : Rat thymus tissue 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 (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 147 kDa **Observed band size:** 180.240 kDa

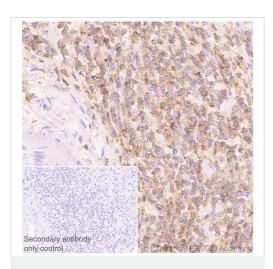
Blocking and diluting buffer and concentration: 5% NFDM/TBST

The MW observed is consistent with the literature (PMID: 14715639).

Negative control: 293T (PMID: 22978632).

This blot was developed using a higher sensitivity ECL substrate.

Exposure time: 3 minutes.

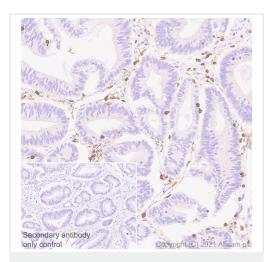


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labelling CD45 with ab281586 at 1/4000 (0.138 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human spleen. The section was incubated with ab281586 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

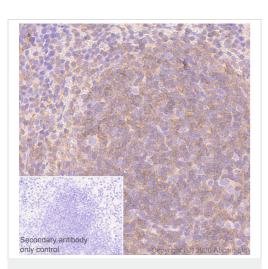


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labelling CD45 with ab281586 at 1/4000 (0.138 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on stromal cells of human colon cancer (PMID: 30713795). The section was incubated with ab281586 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

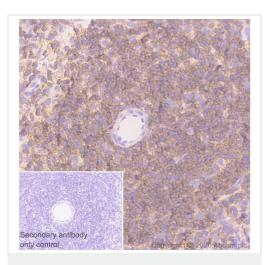


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labelling CD45 with ab281586 at 1/2000 (0.275 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse spleen. The section was incubated with ab281586 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

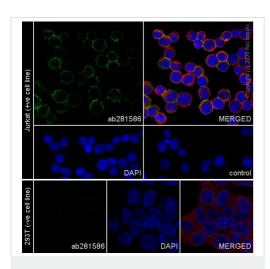


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD45 antibody [RM1007] (ab281586)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labelling CD45 with ab281586 at 1/2000 (0.275 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat spleen. The section was incubated with ab281586 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

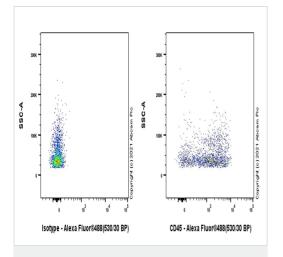


Immunocytochemistry - Anti-CD45 antibody [RM1007] (ab281586)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Jurkat cells labelling CD45 with ab281586 at 1/50 (11.0 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green) Confocal image showing membranouse staining in Jurkat cells and no staining in 293T cells is observed. ab195889 Antialpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

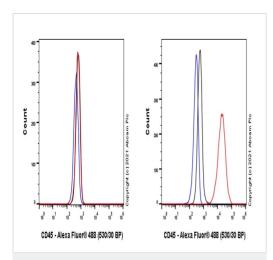
Secondary antibody only control: Secondary antibody is ab150077

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



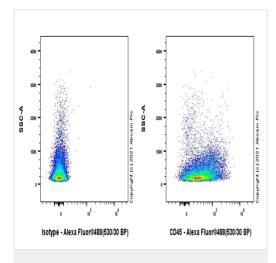
Flow Cytometry (Intracellular) - Anti-CD45 antibody [RM1007] (ab281586)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Human peripheral blood mononuclear cell (PBMC) cells labelling CD45 with ab281586 at 1/500 dilution (0.1ug). Right compared with a Rabbit monoclonal IgG (ab172730)/ Left isotype control. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



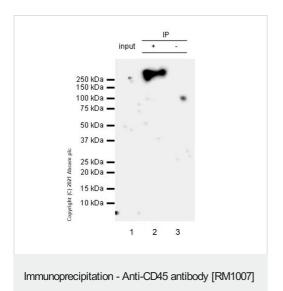
Flow Cytometry (Intracellular) - Anti-CD45 antibody [RM1007] (ab281586)

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 ab281586 at 1/500 dilution (0.1ug) (Red) 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)



Flow Cytometry (Intracellular) - Anti-CD45 antibody [RM1007] (ab281586)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Mouse peripheral blood mononuclear cell (PBMC) cells labelling CD45 with ab281586 at 1/50 dilution (1ug). Right compared with a Rabbit monoclonal lgG (ab172730) / Left isotype control. A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



(ab281586)

CD45 was immunoprecipitated from 0.35 mg Jurkat (human T cell leukemia T lymphocyte), whole cell lysate 10 ug with ab281586 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using 281586 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

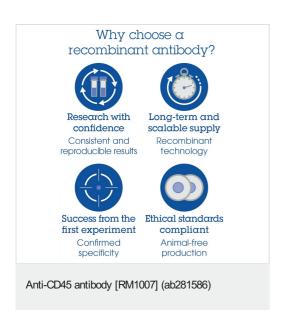
Lane 1: Jurkat (human T cell leukemia T lymphocyte), whole cell lysate 10 ug

Lane 2: ab281586 IP in Jurkat whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of abx in Jurkat whole cell lysate

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

Exposure time: 3 minutes



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