abcam

Product datasheet

Anti-CD45 antibody [MEM-28] ab8216

1 Abreviews 47 References 8 Images

Overview

Product name	Anti-CD45 antibody [MEM-28]
Description	Mouse monoclonal [MEM-28] to CD45
Host species	Mouse
Specificity	Human CD45 antigen (LCA). This antibody reacts with all alternative forms of CD45.
Tested applications	**Suitable for:** Flow Cyt, IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to Human CD45. Human thymocytes and T lymphocytes.
Positive control	IHC-P: Human tonsil tissue. Flow Cyt: Human peripheral blood mononuclear cells. ICC/IF: Human peripheral blood mononuclear cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	MEM-28
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab8216 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
Flow Cyt		Use 1µg for 10^6 cells. (Recognizes an extracellular epitope). **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆ (1)	Use a concentration of 1 µg/ml. Antigen retrieval is not essential but may optimise staining.
WB		Use a concentration of 1 µg/ml. Use under non reducing condition. Predicted molecular weight: 147 kDa.
ICC/IF		Use a concentration of 10 µg/ml. PFA fixation can be used

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.

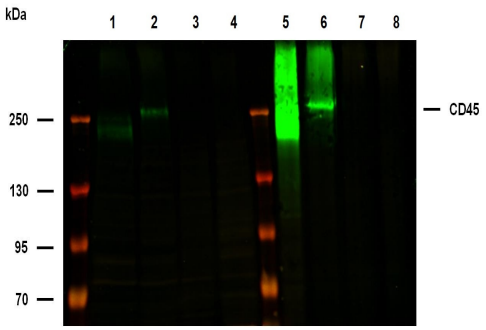
Sequence similarities Belongs to the protein-tyrosine phosphatase family. Receptor class 1/6 subfamily.
Contains 2 fibronectin type-III domains.
Contains 2 tyrosine-protein phosphatase domains.

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 [MEM-28] (ab8216)

All lanes : Anti-CD45 antibody [MEM-28] (ab8216) at 1 µg/ml

Lanes 1 & 5 : Jurkat whole cell extracts with reducing SDS loading buffer
Lane 2 : Raji whole cell extracts with reducing SDS loading buffer
Lane 3 : HeLa whole cell extracts with reducing SDS loading buffer
Lanes 4 & 8 : HEK293T whole cell extracts with non-reducing SDS loading buffer
Lane 6 : Raji whole cell extracts with non-reducing SDS loading buffer
Lane 7 : HeLa whole cell extracts with non-reducing SDS loading buffer

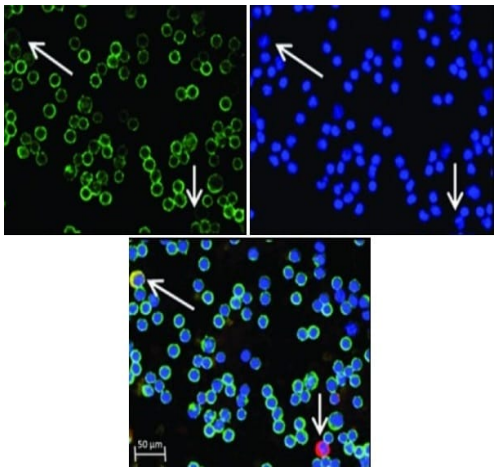
Secondary
All lanes : IRDye 800CW Goat-anti-Mouse IgG

Predicted band size: 147 kDa

Western blotting analysis was performed on whole cell extracts (RIPA lysis buffer) of Jurkat, Raji, HeLa, and HEK293T cell lines, mixed and heated (100°C, 5 min) with reducing and non-reducing SDS-loading buffer. Samples were resolved using 7% Tris-glycine SDS gel electrophoresis.

Nitrocellulose membrane blot was probed with ab8216 (1 µg/ml), followed by IRDye 800CW Goat-anti-Mouse IgG (green). Multiplex fluorescent Western blot detection was performed.

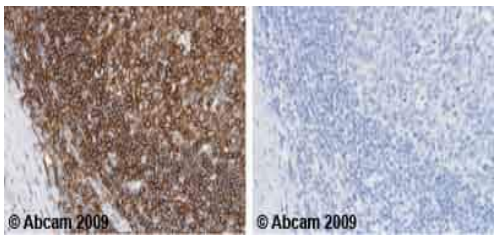
CD45 molecules were detected at ~180-250 kDa in Jurkat and Raji cell lines.



Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [MEM-28] (ab8216)

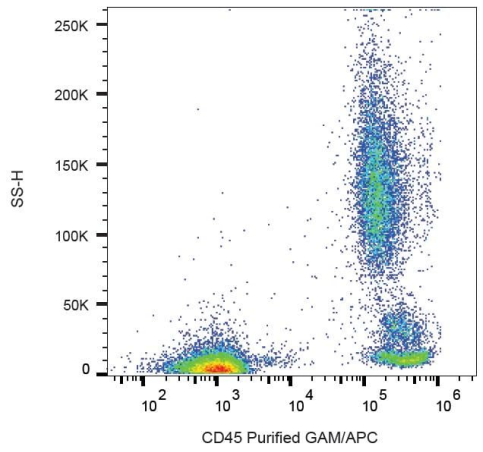
Image from Nel, Ivonne et al. PLoS ONE 11.4 (2016): e0153018. doi: 10.1371/journal.pone.0153018 Fig 3.

Immunocytochemistry/ Immunofluorescence analysis of CTC isolated from mRCC patients stained for hematopoietic cells labeling CD45 with ab8216 (green). The cells were fixed in 4.5% paraformaldehyde for 15 min, washed in PBS, permeabilized with 1x Perm/Wash for 10 min, washing in PBS, blocking of unspecific antibody reactions by incubation with blocking solution containing 5% BSA for 30 min, binding of Anti-CD45 antibody [MEM-28] (ab8216) (final concentration: 5 µg/ml) overnight at 4°C, wash in 0,1% Tween, binding of secondary antibody (Cy3-conjugated goat anti-mouse) for 30 min at 37°C, washing in 0,1% Tween. Subsequently, cells were stained with DAPI for 10 min, mounted with anti-fading medium and stored in the dark until evaluation. Left: CD45, right: DAPI, bottom: merge.



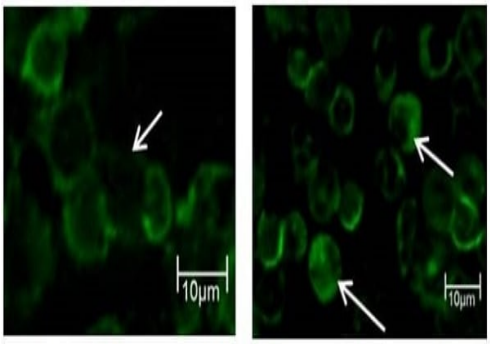
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD45 antibody [MEM-28] (ab8216)

Ab8216 staining human normal tonsil tissue. Staining is localised to cellular membranes.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system (DAKO Autostainer Plus), at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers citrate pH6.1 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



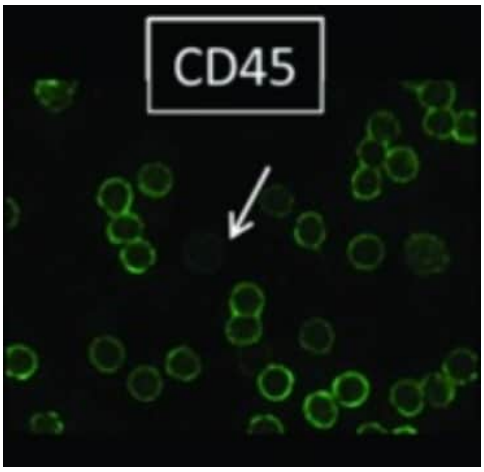
Flow Cytometry - Anti-CD45 antibody [MEM-28] (ab8216)

Flow cytometry analysis (surface staining) of human peripheral blood cells with ab8216 (1 ug/ml), GAM-APC.



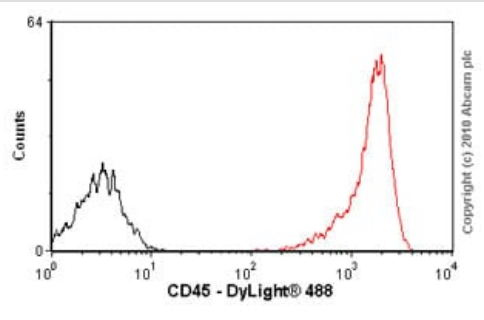
Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [MEM-28] (ab8216)

Immunocytochemistry/ Immunofluorescence analysis of citrated peripheral blood hematologic cells taken from head and neck squamous cell carcinoma (HNSCC) patients labeling CD45 with ab8216 (green). The staining method included fixation of the cells in 4.5% paraformaldehyde for 15 min, washing in PBS, permeabilization with 1× Perm/Wash Buffer for 10 min, washing in PBS, blocking of unspecific antibody reactions by incubation with blocking solution containing 5% BSA for 30 min, binding of Anti-CD45 antibody [MEM-28] (ab8216) (final concentration: 5 µg/ml) overnight at 4°C, wash in 0,1% Tween, binding of Cy3-conjugated secondary antibody for 30 min at 37°C, washing in 0,1% Tween.



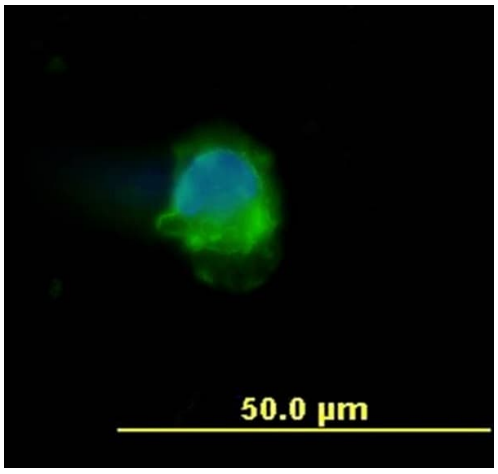
Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [MEM-28] (ab8216)

Immunocytochemistry/ Immunofluorescence analysis of hematopoietic cells labeling CD45 with ab8216. The cells were fixed in 4.5% paraformaldehyde for 15 min, washed in PBS, permeabilized with 1x Perm/Wash for 10 min, washing in PBS, blocking of unspecific antibody reactions by incubation with blocking solution containing 5% BSA for 30 min, binding of Anti-CD45 antibody [MEM-28] (ab8216) (final concentration: 5 µg/ml) overnight at 4°C, wash in 0,1% Tween, binding of secondary antibody (Cy3-conjugated goat anti-mouse) for 30 min at 37°C, washing in 0,1% Tween.



Flow Cytometry - Anti-CD45 antibody [MEM-28] (ab8216)

Overlay histogram showing peripheral blood lymphocytes stained with ab8216 (red line). The cells were incubated with the antibody (ab8216, 1µg/1x10^6 cells) for 30 min at 4ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H&L) (**ab96879**) at 1/200 dilution for 30 min at 4ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed gating on peripheral blood lymphocytes.



Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [MEM-28] (ab8216)

Immunocytochemistry/Immunofluorescence analysis of human peripheral blood mononuclear cells labelling CD45 (green) with ab8216 at 10 µg/mL. Nuclei were counterstained with DAPI (blue).

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