abcam

ab8216

Anti-CD45 antibody [MEM-28]

Anti-CD45 antibody [MEM-28] (ab8216) is a mouse monoclonal antibody detecting CD45 in **Western Blot, Flow Cytometry, IHC-P, ICC/IF**. Suitable for **Human**.

- Over 50 publications
- Trusted since 2001

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.097% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Cell preparation containing PTPRC protein. The exact immunogen used to generate this antibody is proprietary information. Database link P08575
Clone number	MEM-28
Purification technique	Affinity purification Protein A
Specificity	Human CD45 antigen (LCA). This antibody reacts with all alternative forms of CD45.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1 µg/mL
Notes	Antigen retrieval is not essential but may optimise staining.

ICC/IF

Tested

Species	Human
Dilution info	10 µg/mL
Notes	PFA fixation can be used

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	(Recognizes an extracellular epitope). ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Target data

See full target information PTPRC

Function	Protein tyrosine-protein phosphatase required for T-cell activation through the antigen receptor (PubMed:35767951). 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.

Dephosphorylates LYN, and thereby modulates LYN activity (By similarity). Interacts with CLEC10A at antigen presenting cell-T cell contact; CLEC10A on immature dendritic cells recognizes Tn antigen-carrying PTPRC/CD45 receptor on effector T cells and modulates T cell activation threshold to limit autoreactivity. (Microbial infection) Acts as a receptor for human cytomegalovirus protein UL11 and mediates binding of UL11 to T-cells, leading to reduced induction of tyrosine phosphorylation of multiple signaling proteins upon T-cell receptor stimulation and impaired T-cell proliferation.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications
Anti-CD45 antibody [MEM-28] (ab8216) is a mouse monoclonal antibody and is validated for use in Flow Cyt, ICC/IF, IHC-P, WB in human samples.
Anti-CD45 antibody [MEM-28] (ab8216) specifically detects CD45 (UniProt ID: P08575; Molecular weight: 145kDa) and is sold in 100 µg selling sizes.

Quality and Validation
Abcam's high quality validation processes ensure Anti-CD45 antibody [MEM-28] (ab8216) has high sensitivity and specificity.
Anti-CD45 antibody [MEM-28] (ab8216) has been cited over 57 times in peer reviewed journals and is trusted by the scientific community.

Related Products
Antibody clone MEM-28 is also available pre-conjugated to a variety of labels for your convenience - APC, Biotin, PerCP, FITC, PE, PerCP/Cy5.5®, Alexa Fluor® 488, APC/Cy7®, PE/DyLight™ 594, Alexa Fluor® 647, PE/Cy7® (ab28106, ab28107, ab65952, ab134199, ab134202, ab157309, ab187577, ab197130, ab223183, ab239276, ab239317).

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD45 also known as Protein tyrosine phosphatase receptor type C (PTPRC) is a transmembrane glycoprotein with a molecular mass ranging between 180-240 kDa depending on its isoform. It is expressed on the surface of almost all hematopoietic cells except for mature erythrocytes and platelets. CD45 has a critical role in regulating antigen receptor signaling by modifying kinases involved in signal transduction and it is essential in lymphocyte development and activation. Because of its broad expression on immune cells CD45 is a valuable marker for differentiating various immune cell types in assays like flow cytometry and immunohistochemistry (IHC) often referred to as CD45 stain.
Biological function summary	CD45 acts by regulating tyrosine phosphorylation in the immune cell signaling context participating directly in signal transduction. It functions by dephosphorylating specific phosphotyrosine residues on various proteins modulating the signaling threshold required for lymphocyte activation. Although not known to be part of a protein complex CD45 itself shows isoform variation that associates with specific immune cell types impacting their function. Importantly CD45 interacts with multiple signaling molecules to affect cell growth and differentiation.
Pathways	Scientists associate CD45 with signaling pathways such as the T-cell receptor (TCR) and B-cell receptor (BCR) signaling. CD45 modulates the activity of Src family kinases important elements in these pathways making it essential for effective immune response and tolerance. Furthermore interactions between CD45 and proteins like Lck in T-cells or Lyn in B-cells highlight its pivotal role in executing its signaling functions.
Associated diseases and disorders	Scientists often link CD45 to immune-related conditions including autoimmunity and leukemia. In autoimmune diseases altered CD45 expression or activity can disrupt normal immune function contributing to pathogenesis. In leukemia CD45 expression levels can assist in disease classification and prognosis as it interacts with proteins involved in cell cycle regulation. The anti-CD45 antibodies can provide diagnostic and therapeutic avenues highlighting their utility in disease management.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

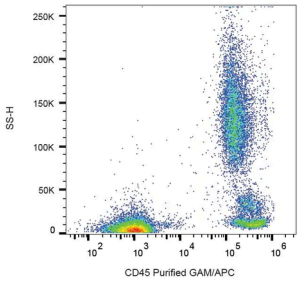
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

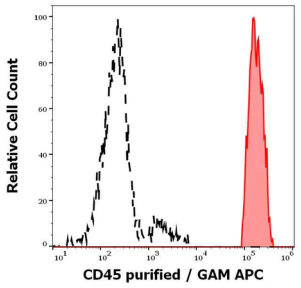
9 product images



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

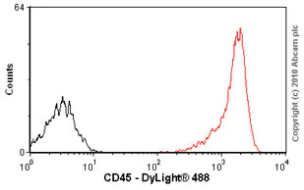
CD45 Flow Cytometry staining using mouse Anti-CD45 antibody

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



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

Separation of human lymphocytes (red-filled) from CD45 negative blood debris (black-dashed) in flow cytometry analysis (surface staining) of human peripheral whole blood stained using ab8216 at 2 µg/ml (GAM APC).



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

CD45 Flow Cytometry staining using mouse Anti-CD45 antibody

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 4°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H&L) (ab96879) at 1/200 dilution for 30 min at 4°C. 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.

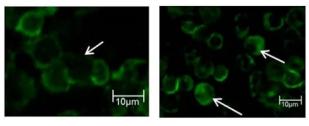


Image from Weller, Patrick et al. PLoS ONE 9.12 (2014): e113706. doi: 10.1371/journal.pone.0113706. Fig 2. Reproduced

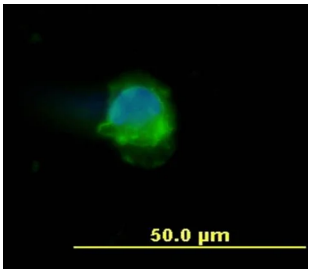
Immunocytochemistry/ Immunofluorescence - Anti-CD45 antibody [MEM-28] (ab8216)

CD45 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-CD45 antibody

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

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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)

CD45 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-CD45 antibody

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

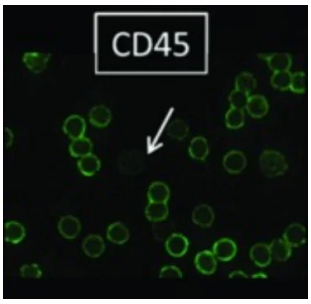


Image from Nel, Ivonne et al. PLoS ONE 11.4 (2016): e0153018. doi: 10.1371/journal.pone.0153018 Fig 2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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.

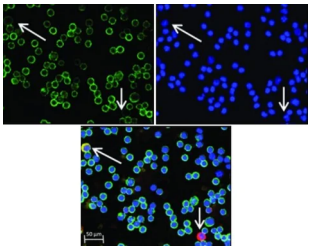
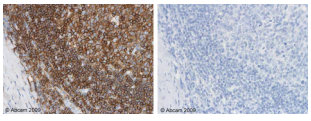


Image from Nel, Ivonne et al. PLoS ONE 11.4 (2016): e0153018. doi: 10.1371/journal.pone.0153018 Fig 3.

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

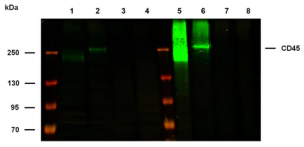
Immunocytochemistry/ Immunofluorescence analysis of CTC isolated from mRCC patients stained for hematopoietic cells labeling CD45 with ab8216 (green). The cells were fixed in 4.5% paraformaldehyde for 15 min, washed in PBS, permeabilized with 1x Perm/Wash for 10 min, washing in PBS, blocking of unspecific 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)

CD45 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-CD45 antibody

ab8216 staining human normal tonsil tissue. Staining is localised to cellular membranes.
Left panel: with primary antibody at 1 µg/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.



Western blot - Anti-CD45 antibody [MEM-28] (ab8216)

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.
All lanes:
Western blot - Anti-CD45 antibody [MEM-28] (ab8216)

Lanes 1 and 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 and 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

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