

DESCRIPTION	
<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human CD45 in direct ELISAs.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 1043759
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived human CD45 protein Gln24-Lys575 Accession # P08575
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

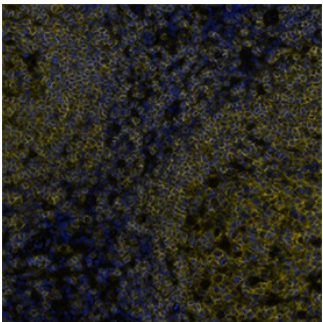
**APPLICATIONS**

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
<b>Western Blot</b>	1 µg/mL	Jurkat human acute T cell leukemia cell line
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	Human PBMC lymphocytes
<b>Multiplex Immunofluorescence</b>	5 µg/mL	Immersion fixed paraffin embedded sections of human lymph node
<b>Immunohistochemistry</b>	5-25 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil
<b>Simple Western</b>	20 µg/mL	Jurkat human acute T cell leukemia cell line
<b>Knockout Validated</b>	CD45 is specifically detected in THP-1 human acute monocytic leukemia parental cell line but is not detectable in CD45 knockout THP-1 human acute monocytic leukemia cell line.	

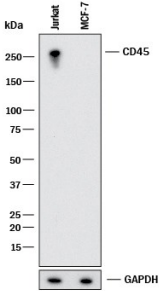
**DATA**

**Multiplex Immunofluorescence**



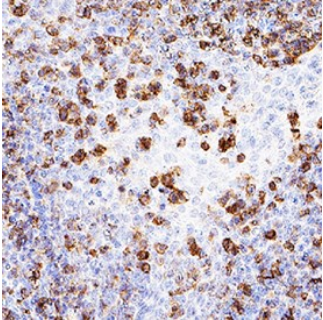
**Detection CD45 in Human Lymph Node via Multiplex Immunofluorescence staining on COMET™** CD45 was detected in immersion fixed paraffin-embedded sections of human lymph node using Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303) at 5µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9). Tissue was stained using the Alexa Fluor™ 555 Goat anti-Mouse IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in COMET™ Panel Builder.

**Western Blot**



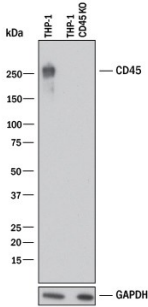
**Detection of Human CD45 by Western Blot.** Western blot shows lysates of Jurkat human acute T cell leukemia cell line and MCF-7 human breast cancer cell line (negative control). PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for CD45 at approximately 255 kDa (as indicated). GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

**Immunohistochemistry**



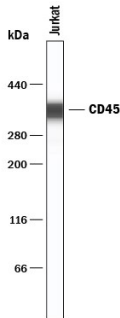
**CD45 in Human Tonsil.** CD45 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUcyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed using our protocol for IHC Staining with VisUcyte HRP Polymer Detection Reagents.

**Knockout Validated**




**Western Blot Shows Human CD45 Specificity by Using Knockout Cell Line.** Western blot shows lysates of THP-1 human acute monocytic leukemia cell line and human CD45 knockout THP-1 human acute monocytic leukemia cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for CD45 at approximately 255 kDa (as indicated) in the parental THP-1 human acute monocytic leukemia cell line, but is not detectable in knockout THP-1 human acute monocytic leukemia cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

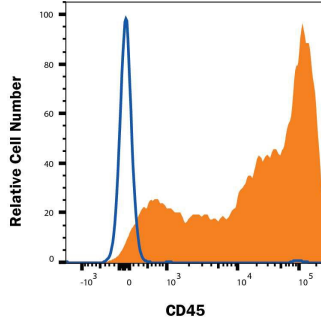
**Simple Western**



**Detection of Human CD45 by Simple Western™.** Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for CD45 at approximately 356 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.



**Flow Cytometry**



**Detection of CD45 in Human Blood Lymphocytes by Flow Cytometry.** Human peripheral blood lymphocytes were stained with Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # MAB14303, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). View our protocol for Staining Membrane-associated Proteins.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
<b>Shipping</b>	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

CD45, previously called LCA (leukocyte common antigen), T200, or Ly5 in mice, is member C of the class 1 (receptor-like) protein tyrosine phosphatase family (PTPRC) (1, 2). It is a variably glycosylated 180-220 kDa transmembrane protein that is abundantly expressed on all nucleated cells of hematopoietic origin (1-3). CD45 has several isoforms, expressed according to cell type, developmental stage and antigenic exposure (1-5). The longest form, CD45RABC (called B220 in mouse), is expressed on B lymphocytes (5). The CD45RABC cDNA encodes 1304 amino acids (aa), including a 23 aa signal sequence, a 552 aa extracellular domain containing the splicing region, a cysteine-rich region and two fibronectin type III domains, a 22 aa transmembrane sequence, and a 707 aa cytoplasmic domain that contains two phosphatase domains, D1 and D2. Only D1 has phosphatase activity. CD45R0 is the shortest form, lacking exons 4, 5 and 6 which encode aa 32-191. It is expressed on memory cells, while intermediate sizes are expressed on other T cells (3, 4, 6). CD45 has been best studied in T cells, where it determines T cell receptor signaling thresholds (3, 6-8). CD45 is moved into or out of the immunological synapse (IS) membrane microdomain depending on the relative influence of interaction with the extracellular galectin lattice or the intracellular actin cytoskeleton (9, 10). Galectin interaction can be fine-tuned by varying usage of the heavily O-glycosylated spliced regions and sialylation of N-linked carbohydrates (4, 9). Within the IS, CD45 dephosphorylates and negatively regulates the Src family kinase, Lck (8-10). In other leukocytes, CD45 influences differentiation and links immunoreceptor signaling with cytokine secretion and cell survival, partially overlapping in function with DEP-1/CD148 (11-14). CD45 deletion causes severe immunodeficiency, while point mutations may be associated with autoimmune disorders (6, 7).

**References:**

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