

## Basic Information

Product Name	Anti-CD45/PTPRC Antibody	
Gene Name	PTPRC	
Source	Rabbit	
Isotype	IgG	
Species Reactivity	human	
Tested Application	WB, IHC, IHC-F, IF, FCM	
Contents	500 ug/ml antibody with PBS , 0.02% NaN <sub>3</sub> , 1 mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human CD45(1214-1254aa EQYQFLYDVIASTYPAQNGQVKNNHQEDKIEFDNEVDKVK), different from the related mouse sequence by eight amino acids, and from the related rat sequence by ten amino acids.	
concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	180-250KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry(Paraffin-embedded Section): 1:50-400 Immunohistochemistry(Frozen Section): 1:50-400 Immunofluorescence (IF): 1:50-400 Flow cytometry (FCM): 1-3 µg/1x10 <sup>6</sup> cells (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

## Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

## Background Information

CD45 (Cluster of Differentiation 45), also known as PTPRC, LCA or CD45R, is an enzyme that, in humans, is encoded by the PTPRC gene. It is a member of the protein tyrosine phosphatase (PTP) family. CD45 is a major high molecular mass leukocyte cell surface molecule which is also an integral membrane protein tyrosine phosphatase. The cytogenetic location of CD45 is 1q31.3-q32.1. This gene is especially a prototype for transmembrane protein-tyrosine phosphatase (PTP). Targeted disruption of the CD45 gene leads to enhanced cytokine and interferon receptor-mediated activation of JAKs and STAT proteins. In vitro, CD45 directly dephosphorylates and binds to JAKs. Functionally, CD45 negatively regulates interleukin-3-mediated cellular proliferation, erythropoietin-dependent hematopoiesis, and antiviral responses in vitro and in vivo. In addition, CD45 has been best studied in T cells, where it determines T cell receptor signaling thresholds. 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. Galectin interaction can be finetuned by varying usage of the heavily Oglycosylated spliced regions and sialylation of Nlinked carbohydrates.

## Selected Validation Data



Figure 1. Western blot analysis of CD45 using anti-CD45 antibody (PB9785). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: JURKAT Whole Cell Lysate, Lane 2: HL-60 Whole Cell Lysate, Lane 3: K562 Whole Cell Lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CD45 antigen affinity purified polyclonal antibody (Catalog # PB9785) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CD45 at approximately 220KD. The expected band size for CD45 is at 220KD.

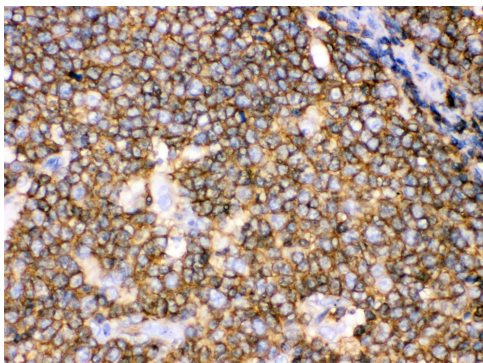


Figure 2. IHC analysis of CD45 using anti-CD45 antibody (PB9785). CD45 was detected in paraffin-embedded section of Human Tonsil Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1µg/ml rabbit anti-CD45 Antibody (PB9785) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

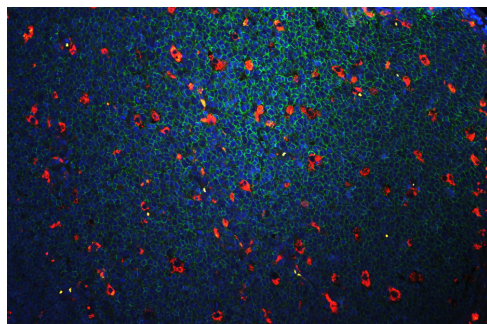


Figure 3. IF analysis of CD68 and CD45 using anti-CD68 antibody (M00602) and anti-CD45 antibody (PB9785)

CD68 and CD45 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution ) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu$ g/mL mouse anti- CD68 Antibody (M00602) and rabbit anti CD45 Antibody(PB9785) overnight at 4°C. DyLight488 Conjugated Goat Anti-Mouse IgG (BA1126) and Biotin conjugated goat anti-rabbit IgG (BA1003) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The tissue section was developed using Cy3 Conjugated Avidin (BA1037). The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.