## Anti-CD45 Antibody [PSH14-19] - BSA and Azide free HA751510

Product Type: Recombinant Rabbit multiclonal IgG, primary antibodies

Species reactivity: Human, Mouse
Applications: WB, IHC-P, IF-Cell

Molecular Wt: Predicted band size: 145 kDa

Clone number: PSH14-19

**Description:** CD45, also known as Protein Tyrosine Phosphatase Receptor Type C (PTPRC), is a

member of the protein tyrosine phosphatase (PTP) family. It is a type I transmembrane protein expressed on the surface of all differentiated hematopoietic cells, except erythrocytes and plasma cells. CD45 plays a crucial role in regulating T-cell and B-cell antigen receptor signaling, which is essential for immune cell activation and function. CD45 consists of an extracellular domain, a single transmembrane segment, and two tandem intracytoplasmic catalytic domains. While only one of the cytoplasmic domains has intrinsic phosphatase activity, both domains are necessary for substrate recruitment. CD45 activates Src family kinases, such as Lck, which are required for T-cell receptor (TCR) signaling. It also modulates the activity of other kinases, including LYN and FYN, and suppresses JAK kinases, thereby acting as a negative regulator of cytokine receptor signaling. CD45 is involved in various cellular processes, including cell growth, differentiation, and immune response regulation. Dysregulation of CD45 function can lead to severe combined immunodeficiency and is implicated in autoimmune diseases, cancer, and infectious diseases. Additionally, CD45 exists in multiple isoforms, which are used in immunohistochemistry to differentiate between immune cell types and diagnose lymphomas and carcinomas.

**Immunogen:** Recombinant protein within mouse CD45 aa 1-566.

Positive control: Jurkat cell lysate, Raji cell lysate, A20 cell lysate, RAW234.7 cell lysate, Jurkat, RAW264.7,

human lymph nodes tissue, mouse spleen tissue.

Subcellular location: Cell membrane, Membrane raft, Synapse.

Database links: SwissProt: P08575 Human | P06800 Mouse

**Recommended Dilutions:** 

 WB
 1:5,000

 IHC-P
 1:1,000

 IF-Cell
 1:500-1:1,000

 Storage Buffer:
 PBS (pH7.4).

**Storage Instruction:** Store at  $+4^{\circ}$ C after thawing. Aliquot store at  $-20^{\circ}$ C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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## **Images**

 **Fig1:** Western blot analysis of CD45 on different lysates with Rabbit anti-CD45 antibody (HA751510) at 1/5,000 dilution.

Lane 1: Jurkat cell lysate

Lane 2: MCF7 cell lysate (negative)

Lane 3: Raji cell lysate Lane 4: A20 cell lysate

Lane 5: C2C12 cell lysate (negative)

Lane 6: RAW234.7 cell lysate

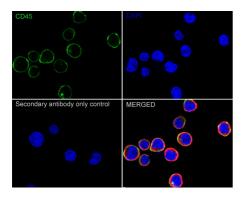
Lysates/proteins at 20 µg/Lane.

Predicted band size: 145 kDa Observed band size: 250 kDa

Exposure time: 20 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751510) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunocytochemistry analysis of Jurkat cells labeling CD45 with Rabbit anti-CD45 antibody (HA751510) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD45 antibody (HA751510) at 1/500 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor  $\pm$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



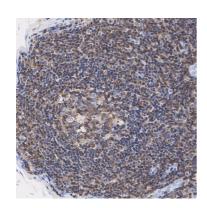
Secondary antibody only control

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**Fig3:** Immunocytochemistry analysis of RAW264.7 cells labeling CD45 with Rabbit anti-CD45 antibody (HA751510) at 1/1,000 dilution.

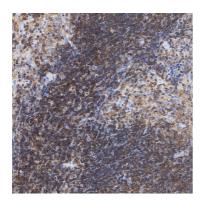
Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD45 antibody (HA751510) at 1/1,000 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



**Fig4:** Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD45 antibody (HA751510) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA751510) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD45 antibody (HA751510) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA751510) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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## **Background References**

- 1. Katagiri T, et al. CD45 negatively regulates lyn activity by dephosphorylating both positive and negative regulatory tyrosine residues in immature B cells. J Immunol. 1999, Aug.
- 2. Holmes N. CD45: all is not yet crystal clear. Immunology. 2006, Feb.