Anti-CD45 Antibody [JE03-05]

ET7111-03

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, FC, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 147 kDa.
Clone number:	JE03-05

Description: Protein tyrosine phosphatase, receptor type, C also known as PTPRC is an enzyme that, in humans, is encoded by the PTPRC gene. PTPRC is also known as CD45 antigen (CD stands for cluster of differentiation), which was originally called leukocyte common antigen (LCA). The protein product of this gene, best known as CD45, is a member of the protein tyrosine phosphatase (PTP) family. PTPs are signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. CD45 contains an extracellular domain, a single transmembrane segment, and two tandem intracytoplasmic catalytic domains, and thus belongs to the receptor type PTP family. CD45 is a type I transmembrane protein that is present in various isoforms on all differentiated hematopoietic cells (except erythrocytes and plasma cells). CD45 has been shown to be an essential regulator of T- and B-cell antigen receptor signaling. It functions through either direct interaction with components of the antigen receptor complexes via its extracellular domain (a form of co-stimulation), or by activating various Src family kinases required for the antigen receptor signaling via its cytoplasmic domain. CD45 also suppresses JAK kinases, and so functions as a negative regulator of cytokine receptor signaling.

Immunogen: Recombinant protein within Human CD45 aa 1,210-1,306 / 1,306.

Positive control: Jurkat cell lysates, human colon cancer tissue, human tonsil tissue, human spleen tissue, Jurkat.

Subcellular location: Cell junction, Cell membrane, Membrane

Database links: SwissProt: P08575 Human

Recommended Dilutions:

WB	1:500-1:1000
IHC-P	1:1,000
FC	1:50-1:100
IF-Tissue	1:500
mIHC	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

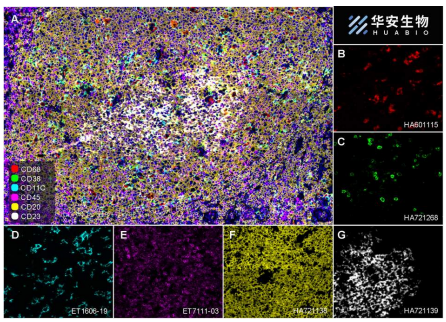

Fig1: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (HA601115, Red), anti-CD38 (HA721268, Green), anti-CD23 (HA721139, White), anti-CD11C (ET1606-19, Cyan), anti-CD45 (ET7111-03, Magenta) and anti-CD20 (HA721138, Yellow) on tonsil. Panel B: anti-CD68 stained on Macrophage. Panel C: anti-CD38 stained on lymphocyte subsets. Panel D: anti-CD11C stained on dendritic cells. Panel E: CD45 stained on lymphocytes. Panel F: anti-CD20 stained on B cells. Panel G: anti-CD23 stained on follicular dendritic cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of HA601115 (1/2,000 dilution), HA721268 (1/1,000 dilution), ET1606-19 (1/1,000 dilution), ET7111-03 (1/500 dilution), HA721138 (1/2,000 dilution) and HA721139 (1/800 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

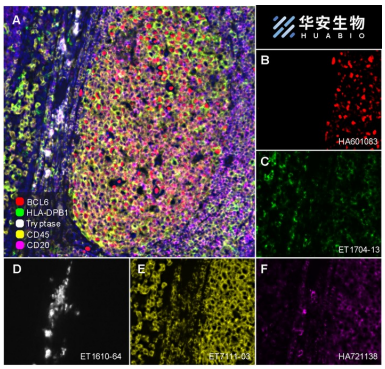

Fig2: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-BCL6 (HA601083, Red), anti-HLA-DPB1 (ET1704-13, Green), anti-Tryptase (ET1610-64, White), anti-CD20 (HA721138, Magenta) and anti-CD45 (ET7111-03, Yellow) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA601083 (1/200 dilution), ET1704-13 (1/2,000 dilution), ET1610-64 (1/5,000 dilution), HA721138 (1/2,000 dilution) and ET7111-03 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

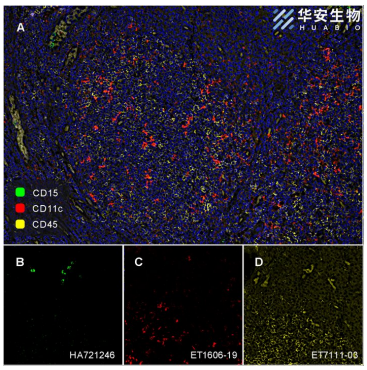

Fig3: Fluorescence multiplex immunohistochemical analysis of human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD45 (ET7111-03, Yellow), anti-CD15 (HA721246, Green) and anti-CD11c (ET1606-19, Red) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET7111-03 (1/500 dilution), HA721246 (1/500 dilution) and ET1606-19 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

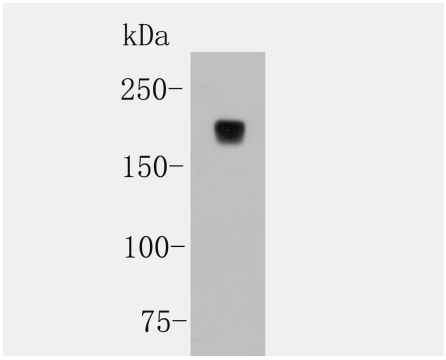

Fig4: Western blot analysis of CD45 on Jurkat cell lysates with Rabbit anti-CD45 antibody (ET7111-03) at 1/1000 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 147 kDa
Observed band size: 200 kDa

Exposure time: 2 minutes;

8% 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 (ET7111-03) at 1/1000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

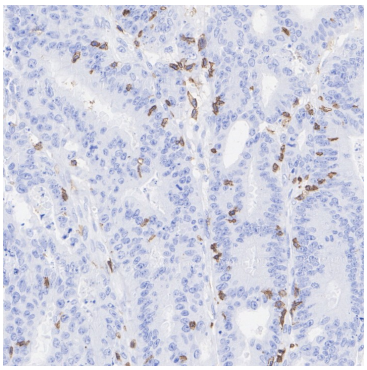

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-CD45 antibody (ET7111-03) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET7111-03) 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.

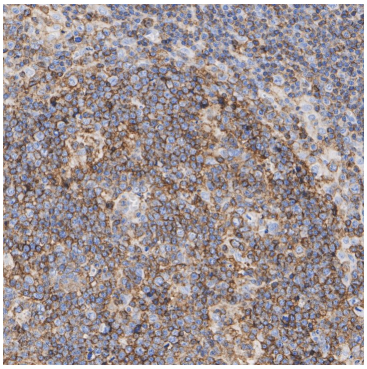

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD45 antibody (ET7111-03) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET7111-03) at 1/200 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.

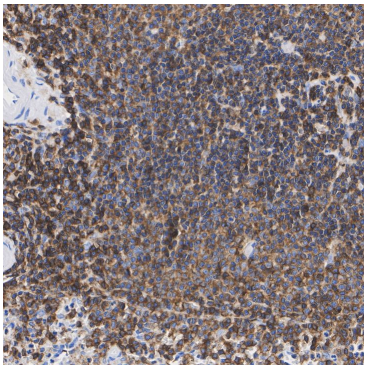

Fig7: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD45 antibody (ET7111-03) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET7111-03) at 1/200 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.

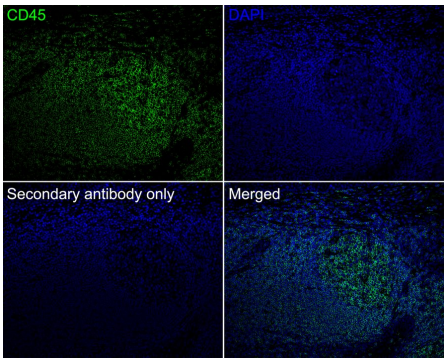

Fig8: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling CD45 with Rabbit anti-CD45 antibody (ET7111-03) at 1/500 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET7111-03, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

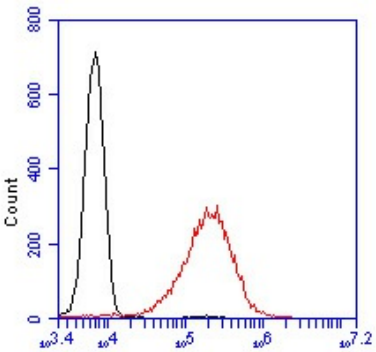

Fig9: Flow cytometric analysis of CD45 was done on Jurkat cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7111-03, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Gabaev I.et.al.The human cytomegalovirus UL11 protein interacts with the receptor tyrosine phosphatase CD45, resulting in functional paralysis of T cells.PLoS Pathog. 7:E1002432-E1002432(2011).