Anti-CD4 Antibody [ST0488]

ET1609-52

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, mIHC
Molecular Wt:	Predicted band size: 51 kDa
Clone number:	ST0488

Description: The T cell receptor (TCR) is a heterodimer composed of either α and β or γ and δ chains. CD3 chains and the CD4 or CD8 co-receptors are also required for efficient signal transduction through the TCR. The TCR is expressed on T helper and T cytotoxic cells that can be distinguished by their expression of CD4 and CD8; T helper cells express CD4 proteins and T cytotoxic cells display CD8. CD4 is also expressed on cortical cells, mature medullary thymocytes, microglial cells and dendritic cells. CD4 (also designated T4 and Leu 3), is a membrane glycoprotein that contains four extracellular immunoglobin-like domains. The TCR in association with CD4 can bind class II MHC molecules presented by the antigen-presenting cells. The CD4 protein functions by increasing the avidity of the interaction between the TCR and an antigen-class II MHC complex. An additional role of CD4 is to function as a receptor for HIV.

Immunogen: Recombinant protein within Human CD4 aa 196-416 / 458.

Positive control: U937 cell lysate, THP-1 cell lysate, THP-1, human tonsil tissue, human spleen tissue, human lymph nodes tissue, human liver tissue, human prostate cancer, human cervical cancer.

Subcellular location: Cell membrane.

Database links: SwissProt: P01730 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:200
IHC-P	1:400-1:800
FC	1:500-1:1,000
mIHC	1:800-1:1,000

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

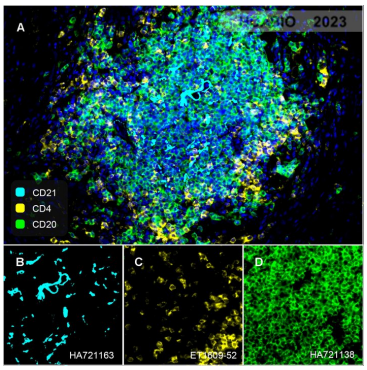

Fig1: Fluorescence multiplex immunohistochemical analysis of tertiary lymphoid structures in human prostate cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD21 (HA721163, cyan) and anti-CD4 (ET1609-52, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-CD21 stained on naive B-cell, memory B-cell and plasma cells. Panel D: anti-CD4 stained on helper T cells and Treg 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 three rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721163 (1/1,000 dilution), and ET1609-52 (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 Olympus VS200 Slide Scanner.

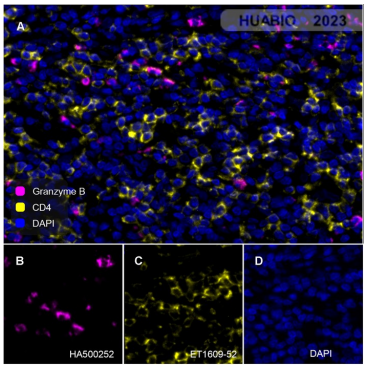

Fig2: Fluorescence multiplex immunohistochemical analysis of tertiary lymphoid structures in human cervical cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Granzyme B (HA500252, magenta), anti-CD4 (ET1609-52, yellow) on tertiary lymphoid structures. Panel B: anti- Granzyme B stained on cytotoxic NK cells and dendritic cells. Panel C: anti-CD4 stained on helper T cells and Treg 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 three rounds of staining: in the order of HA500252 (1/200 dilution), ET1609-52 (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 Olympus VS200 Slide Scanner.

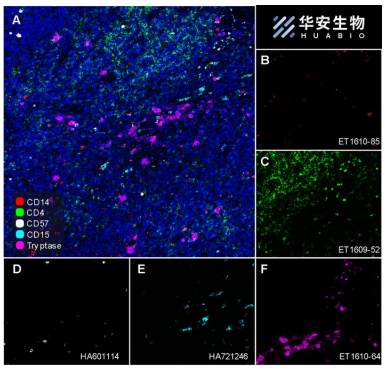

Fig3: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD14 (ET1610-85, Red), anti-CD4 (ET1609-52, Green), anti-CD57 (HA601114, White), anti-CD15 (HA721246, Cyan)and anti-Tryptase (ET1610-64, Magenta) on tonsil. Panel B: anti- CD14 stained on monocytes. Panel C: anti-CD4 stained on helper T cells and Treg cells. Panel D: anti-CD57 stained on NK cells and T cells. Panel E: CD15 stained on granulocytes and monocytes. Panel F: anti-Tryptase stained on Mast 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 five rounds of staining: in the order of ET1610-85 (1/800 dilution), ET1609-52 (1/800 dilution), HA601114 (1/1,000 dilution), HA721246 (1/500 dilution), and ET1610-64 (1/3,000 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 Olympus VS200 Slide Scanner.

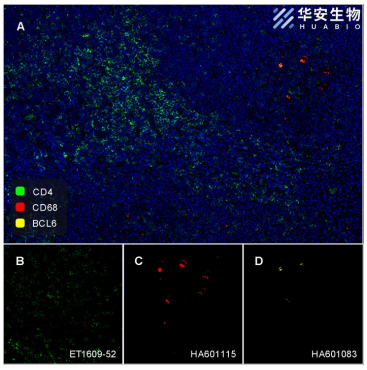

Fig4: Fluorescence multiplex immunohistochemical analysis of human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (HA601115, Red), anti-BCL6 (HA601083, Yellow) and anti-CD4 (ET1609-52, Green) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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 HA601115 (1/2,000 dilution), HA601083 (1/200 dilution) and ET1609-52 (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 Zeiss Observer 7 Inverted Fluorescence Microscope.

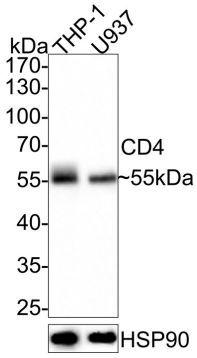

Fig5: Western blot analysis of CD4 on different lysates with Rabbit anti-CD4 antibody (ET1609-52) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: U937 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 51 kDa
Observed band size: 55 kDa

Exposure time: 1 minute 30 seconds;

10% 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 (ET1609-52) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

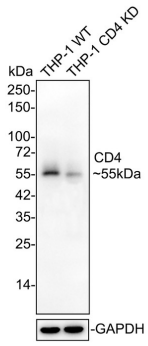

Fig6: Western blot analysis of CD4 on different lysates with Rabbit anti-CD4 antibody (ET1609-52) at 1/2,000 dilution.

Lane 1: THP-1 WT cell lysate
Lane 2: THP-1 CD4 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 51 kDa
Observed band size: 55 kDa

Exposure time: 30 seconds;
ECL: Ori Supersensitive

4-20% SDS-PAGE gel.

ET1609-52 was shown to specifically react with CD4 in THP-1 WT cells. Weakened band was observed when THP-1 CD4 KD sample was tested. THP-1 WT and THP-1 CD4 KD samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1609-52, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

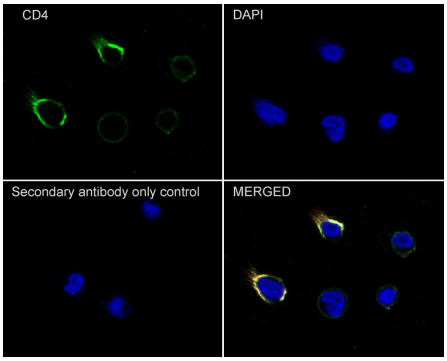

Fig7: Immunocytochemistry analysis of THP-1 cells labeling CD4 with Rabbit anti-CD4 antibody (ET1609-52) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-CD4 antibody (ET1609-52) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

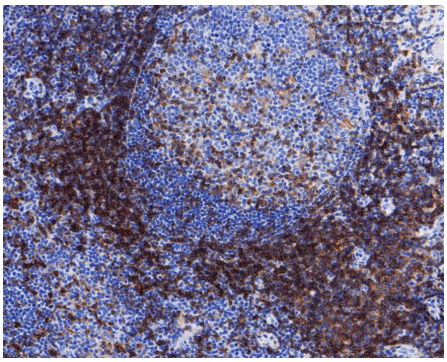

Fig8: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD4 antibody (ET1609-52) at 1/800 dilution.

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 (ET1609-52) at 1/800 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.

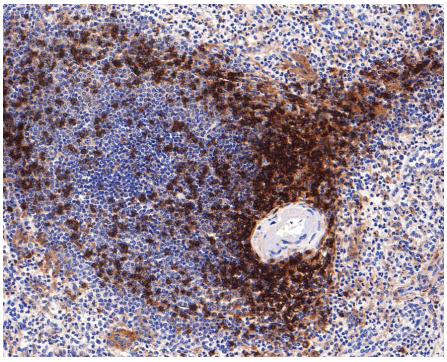

Fig9: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD4 antibody (ET1609-52) at 1/400 dilution.

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 (ET1609-52) at 1/400 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.

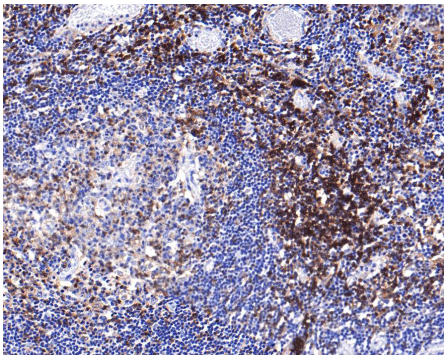

Fig10: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD4 antibody (ET1609-52) at 1/400 dilution.

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 (ET1609-52) at 1/400 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.

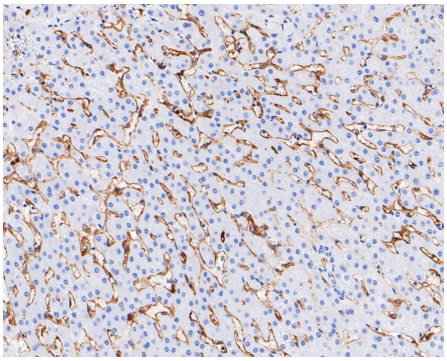

Fig11: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-CD4 antibody (ET1609-52) at 1/800 dilution.

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 (ET1609-52) at 1/800 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.

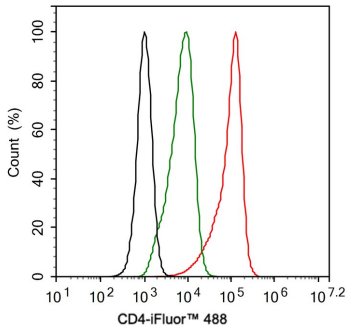

Fig12: Flow cytometric analysis of THP-1 cells labeling CD4.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (ET1609-52, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

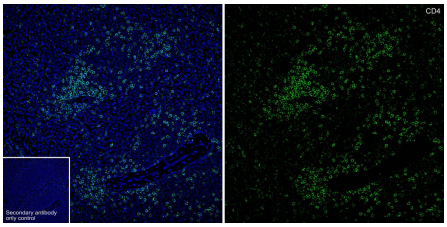

Fig13: Application: IF-Tissue

Species: Human

Site: Spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/200

Background References

1. Kim EJ et al. Costimulation blockade alters germinal center responses and prevents antibody-mediated rejection. Am J Transplant 14:59-69 (2014).
2. Liu XD et al. Resistance to Antiangiogenic Therapy Is Associated with an Immunosuppressive Tumor Microenvironment in Metastatic Renal Cell Carcinoma. Cancer Immunol Res 3:1017-29 (2015).