Anti-CD8 alpha Antibody [SI18-01]

ET1606-31

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	SI18-01

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 (CD8-α and CD8-β) 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 proteins; T helper cells express CD4 proteins and T cytotoxic cells display CD8 proteins. CD8s are cell surface glycoproteins that exist as two chain complex (αα or αβ) receptors that bind class I MHC molecules presented by the antigen-presenting cell (APC). A primary function of CD8 proteins is to facilitate antigen recognition by the TCR and to strengthen the avidity of the TCR-antigen interactions. An additional role for CD8-expressing T cells may be to maintain low levels of HIV expression.

Immunogen: Synthetic peptide within Human CD8 alpha aa 186-235 / 235.

Positive control: THP-1 cell lysate, Jurkat cell lysate, PC-3M cell lysate, Hela, CRC, human spleen tissue, human lymph node tissue, human appendix tissue, Jurkat.

Subcellular location: Cell membrane, Secreted.

Database links: SwissProt: P01732 Human

Recommended Dilutions:

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

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

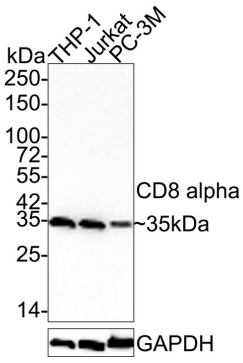

Fig1: Western blot analysis of CD8 alpha on different lysates with Rabbit anti-CD8 alpha antibody (ET1606-31) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: PC-3M cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 26 kDa
Observed band size: 35 kDa

Exposure time: 2 minutes;

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 (ET1606-31) at 1/2,000 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.

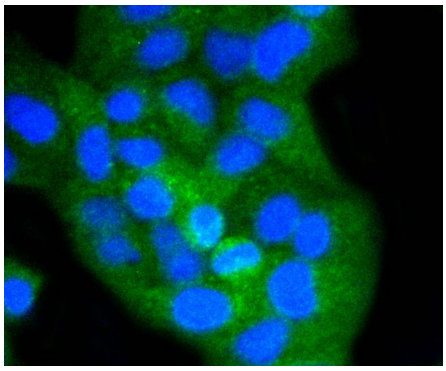

Fig2: ICC staining of CD8 alpha in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1606-31, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

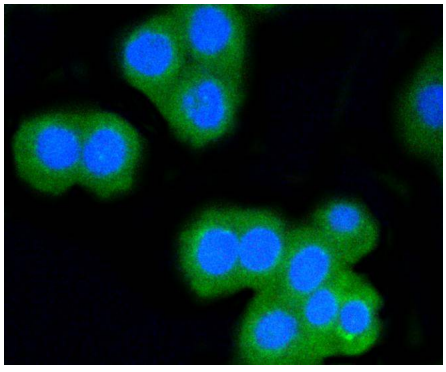

Fig3: ICC staining of CD8 alpha in CRC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1606-31, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

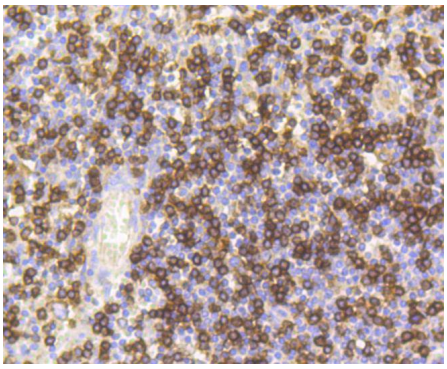

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-CD8 alpha antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-31, 1/50) for 30 minutes 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.

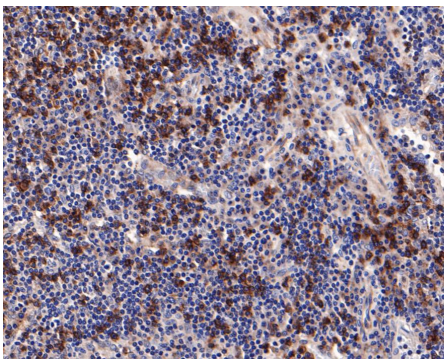

Fig5: Immunohistochemical analysis of paraffin-embedded human lymph node tissue with Rabbit anti-CD8 alpha antibody (ET1606-31) at 1/100 dilution.

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 (ET1606-31) at 1/100 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.

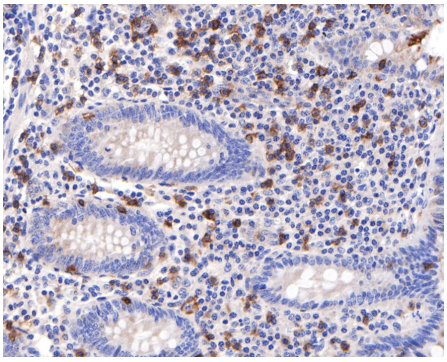

Fig6: Immunohistochemical analysis of paraffin-embedded human appendix tissue with Rabbit anti-CD8 alpha antibody (ET1606-31) at 1/100 dilution.

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 (ET1606-31) at 1/100 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.

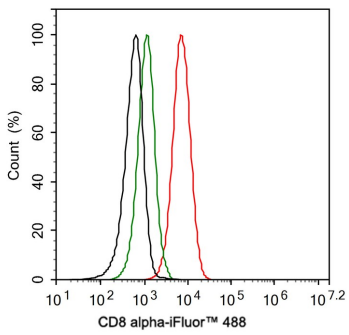

Fig7: Flow cytometric analysis of Jurkat cells labeling CD8 alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1606-31, 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).

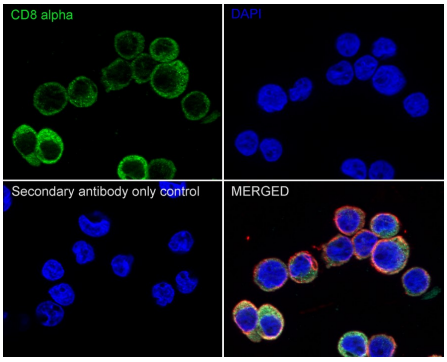

Fig8: Immunocytochemistry analysis of THP-1 cells labeling CD8 alpha with Rabbit anti-CD8 alpha antibody (ET1606-31) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-CD8 alpha antibody (ET1606-31) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (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.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Zehbe, I. et al. 2007. Human papillomavirus 16 E6-specific CD45RA+ CCR7+ high avidity CD8+ T cells fail to control tumor growth despite interferon-gamma production in patients with cervical cancer. J Immunother. 30: 523-532.
2. Morales, P.J. et al. 2003. Placental cell expression of HLA-G2 isoforms is limited to the invasive trophoblast phenotype. J. Immunology. 171: 6215-6224.