

Anti-CD3 epsilon Antibody [SY0239]

ET1607-29



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, FC, IP
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	SY0239

Description: The T cell antigen receptor (TCR) recognizes foreign antigens and translates such recognition events into intracellular signals that elicit a change in the cell from a dormant to an activated state. Much of this signaling process can be attributed to a multisubunit complex of proteins that associates directly with the TCR. This complex has been designated CD3 (cluster of differentiation 3). CD3 is composed of four polypeptides: ζ , γ , ϵ and δ . Each of these polypeptides contains at least one immunoreceptor tyrosine-based activation motif (ITAM). Engagement of TCR complex with foreign antigens induces tyrosine phosphorylation in the ITAM motifs and phosphorylated ITAMs function as docking sites for signaling molecules such as ZAP-70 and p85 subunit of PI-3 kinase. TCR ligation also induces a conformational change in CD3 ϵ , such that a proline region is exposed and then associates with the adaptor protein Nck.

Immunogen: Synthetic peptide within human CD3 epsilon aa 153-203 (Cytoplasmic).

Positive control: Human thymus tissue lysate, Jurkat cell lysate, human tonsil tissue, human spleen tissue, Jurkat.

Subcellular location: Cell membrane.

Database links: SwissProt: P07766 Human

Recommended Dilutions:

WB	1:500-1:2,000
IHC-P	1:50-1:1,000
IF-Cell	1:50-1:100
IF-Tissue	1:1,000
FC	1:1,000
IP	Use at an assay dependent concentration.

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

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

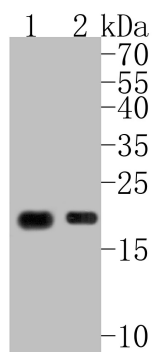


Fig1: Western blot analysis of CD3 epsilon on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1607-29, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: Human thymus tissue lysate

Lane 2: Jurkat cell lysate

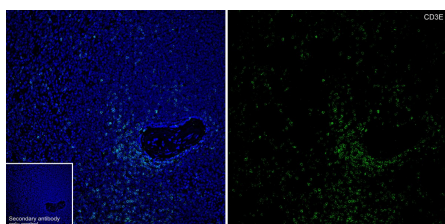


Fig2: Application: IF-Tissue

Species: Human

Site: spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

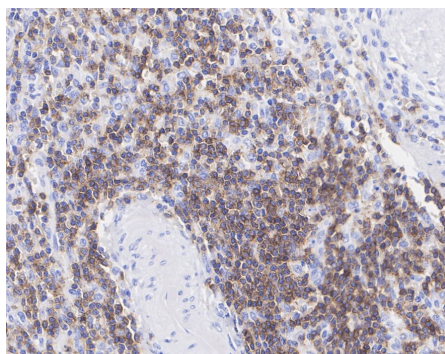


Fig3: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD3 epsilon antibody (ET1607-29) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1607-29) 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.

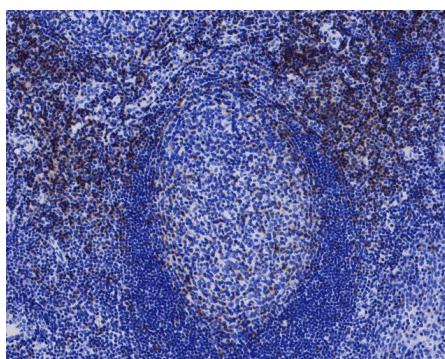


Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-CD3 epsilon antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1607-29, 1/200) 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.

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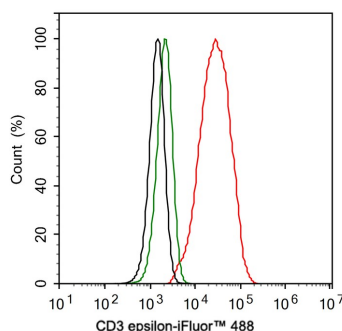


Fig5: Flow cytometric analysis of Jurkat cells labeling CD3 epsilon.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1607-29, 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).

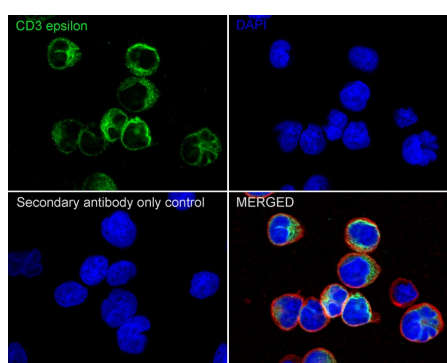


Fig6: Immunocytochemistry analysis of Jurkat cells labeling CD3 epsilon with Rabbit anti-CD3 epsilon antibody (ET1607-29) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, 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-CD3 epsilon antibody (ET1607-29) at 1/50 dilution in 2% negative goat serum overnight at 4 °C. 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.

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

Background References

1. Huang YH et al. CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. *Nature* 517:386-90 (2015).
2. Berglin L et al. In situ characterization of intrahepatic non-parenchymal cells in PSC reveals phenotypic patterns associated with disease severity. *PLoS One* 9:e105375 (2014).

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