

# Anti-CD3 Antibody [JE80-02]

HA720082



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human
<b>Applications:</b>	WB, IHC-P, IF-Tissue, IP, mIHC, IF-Cell
<b>Molecular Wt:</b>	Predicted band size: 23 kDa
<b>Clone number:</b>	JE80-02

**Description:** The CD3 protein is a T-cell marker, a complex of four structurally distinct membrane glycoprotein isoforms, 20-50 kDa, comprising extracellular, transmembrane and intracellular domains. The CD3 complex is responsible for mediating signal transduction to the internal environment upon antigenic recognition by TCR, causing T-cell proliferation and release of cytokines. Except for a weak expression in Purkinje cells (with some of the Abs) and activated NK-cells, CD3 is found only in T-cells. CD3 appear in the cytoplasm of prothymocytes, and on the surface of about 95% of thymocytes, while cytoplasmic CD3 is lost as the cells differentiate into medullary thymocytes. In therapy resistant celiac disease, a shift from membranous to cytoplasmic CD3 expression is seen (together with loss of CD8). In malignant lymphomas, CD3 is a pan-T-cell lineage-restricted antigen, detected in 80-97% of the T-cell lymphomas. Mature T-cell lymphomas including cases of mycosis fungoides, peripheral T-cell lymphoma and anaplastic large cell lymphoma may aberrantly lose CD3. NK-cell lymphomas can show a cytoplasmic reaction. Reed-Sternberg cells may show a globular paranuclear reaction. CD3 is an important marker in the classification of malignant lymphomas and lymphoid leukaemias. Also the marker is useful for the identification of T-cells in, e.g., celiac disease, lymphocytic colitis and colorectal carcinomas associated with loss of a mismatch repair protein.

**Immunogen:** Synthetic peptide within human CD3E aa 158-207/207.

**Positive control:** Jurkat cell lysates, human lymph nodes tissue, human spleen tissue, human small cell lung cancer, human non-small cell lung cancer, human gastric cancer.

**Subcellular location:** Cell membrane.

**Database links:** SwissProt: P07766 Human | P04234 Human | P09693 Human | P20963 Human

**Recommended Dilutions:**

<b>WB</b>	1:1,000-1:2,000
<b>IHC-P</b>	1:600-1:1,000
<b>IF-Tissue</b>	1:100-1:200
<b>IP</b>	Use at an assay dependent concentration.
<b>mIHC</b>	1:500
<b>IF-Cell</b>	1:100

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

**Storage Instruction:** Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

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Technical:0086-571-89986345

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**Fig1:** Western blot analysis of CD3 on Jurkat cell lysates with Rabbit anti-CD3 antibody (HA720082) at 0.5 $\mu$ g/ml dilution.

Lysates/proteins at 10  $\mu$ g/Lane.

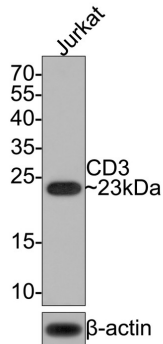
Predicted band size: 23 kDa

Observed band size: 23 kDa

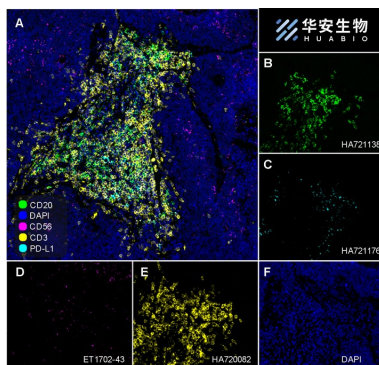
Exposure time: 2 minutes;

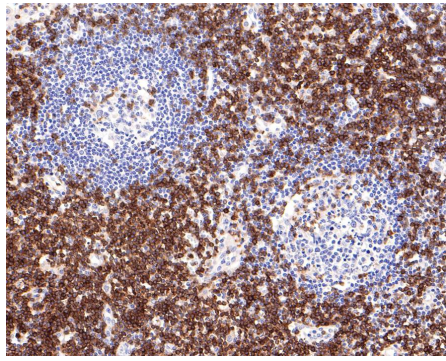
15% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA720082) at 0.5 $\mu$ g/ml dilution was used in 5% NFDN/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.



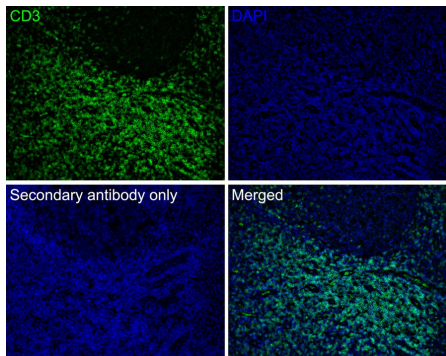
**Fig2:** Fluorescence multiplex immunohistochemical analysis of Tertiary Lymphoid Structures in Human Small Cell Lung Cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-PD-L1 (HA721176, cyan), anti-CD56 (ET1702-43, magenta) and anti-CD3 (HA720082, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel D: anti-CD56 stained on NKT cells. Panel E: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721176 (1/1,000 dilution), ET1702-43 (1/1,000 dilution), and HA720082 (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 $^{\circ}$ C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.





**Fig3:** Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD3 antibody (HA720082) at 1/600 dilution.

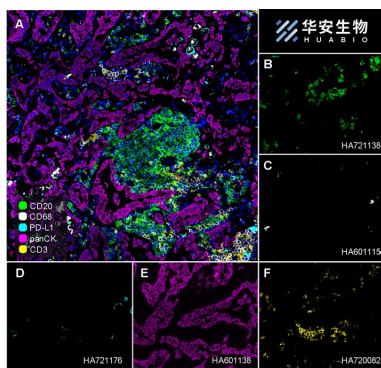
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA720082) at 1/600 dilution for 1 hour 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.



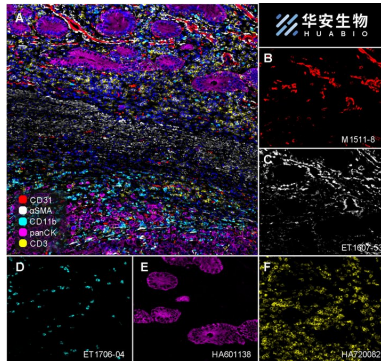
**Fig4:** Immunofluorescence analysis of paraffin-embedded human lymph nodes tissue labeling CD3 with Rabbit anti-CD3 antibody (HA720082) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA720082, green) at 1/100 dilution overnight at 4 °C, washed with PBS.

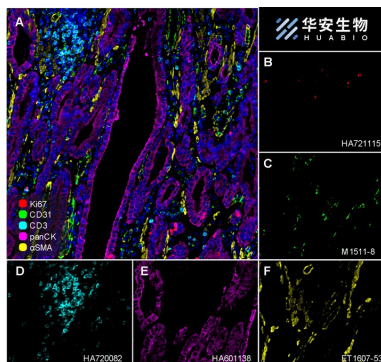
Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).



**Fig5:** Fluorescence multiplex immunohistochemical analysis of the human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD68 (HA601115, gray), anti-PD-L1 (HA721176, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human non-small cell lung cancer. Panel B: anti-CD20 stained on B cells. Panel C: anti-CD68 stained on macrophage M1 and macrophage M2. Panel D: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA721138 (1/1,500 dilution), HA601115 (1/2,000 dilution), HA721176 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner

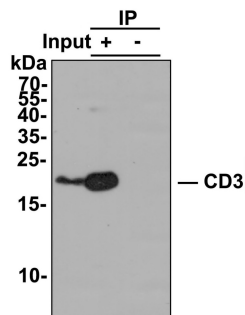


**Fig6:** Fluorescence multiplex immunohistochemical analysis of the human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD31 (M1511-8, red), anti- $\alpha$ SMA (ET1607-53, gray), anti-CD11b (ET1706-04, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human gastric cancer. Panel B: anti-CD31 stained on the endothelial cells. Panel C: anti- $\alpha$ SMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel D: anti-CD11b stained on myeloid cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of M1511-8 (1/1,000 dilution), ET1607-53 (1/2,000 dilution), ET1706-04 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.



**Fig7:** Fluorescence multiplex immunohistochemical analysis of the human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Ki67 (HA721115, red), anti-CD31 (M1511-8, green), anti-CD3 (HA720082, cyan), anti-panCK (HA601138, magenta) and anti- $\alpha$ SMA (ET1607-53, yellow) on human gastric cancer. Panel B: anti- Ki67 stained on cells in G1, S, G2 and M phases of cell cycle. Panel C: anti-CD31 stained on the endothelial cells. Panel D: anti-CD3 stained on T cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti- $\alpha$ SMA stained on cancer-associated fibroblasts and smooth muscle cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA721115 (1/2,000 dilution), M1511-8 (1/1,000 dilution), HA720082 (1/500 dilution), HA601138 (1/3,000 dilution), and ET1607-53 (1/2,000 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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.





**Fig8:** CD3 was immunoprecipitated from 1mg/ml Jurkat whole cell lysate with HA720082 at 2ug/ml dilution. Western blot was performed from the immunoprecipitate using HA720082 at 1ug/ml dilution. Goat anti-Rabbit IgG-HRP antibody (HA1001) was used as the secondary antibody at 1/300,000 dilution.

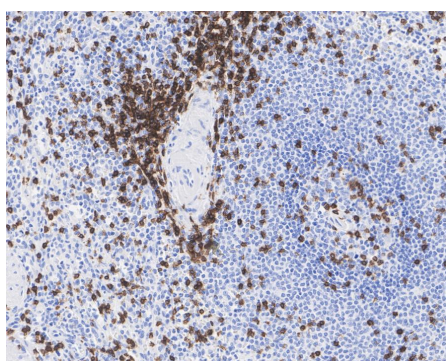
Lane 1: Jurkat whole cell lysate 5  $\mu$ g (Input).

Lane 2: HA720082 IP in Jurkat whole cell lysate.

Lane 3: Rabbit monoclonal IgG instead of HA720082 in Jurkat whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFD/MTBST.

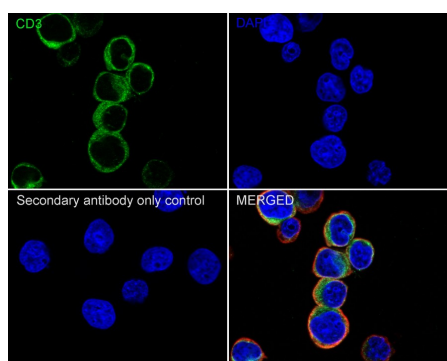
Exposure time: 30 seconds.



**Fig9:** Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD3 antibody (HA720082) 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 (HA720082) 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.

**Fig10:** Immunocytochemistry analysis of Jurkat cells labeling CD3 with Rabbit anti-CD3 antibody (HA720082) 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-CD3 antibody (HA720082) at 1/100 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor<sup>TM</sup> 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  $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor<sup>TM</sup> 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".

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