Anti-CD11b Antibody [JU93-81]

ET1706-04

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IP, IHC-P, FC, IF-Cell, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 127 kDa
Clone number:	JU93-81

Description: Integrin αM, also designated complement component receptor-3 α, CD11b (p170), macrophage antigen a polypeptide, cell surface glycoprotein Mac-1 a subunit, MAC1A, MO1A and ITGAM) is a cell adhesion molecule that acts as a receptor for cell surface ligands such as intracellular adhesion molecules (ICAMs) or soluble ligands. Integrins are heterodimeric proteins that contain an a chain and b chain. Integrin αM combines with the Integrin β2 to form a leukocyte-specific integrin referred to as macrophage receptor 1 (Mac-1), or inactivated-C3b (iC3b) receptor 3 (CR3). Integrin αM/β2 is important in the adherence of neutrophils and monocytes to stimulated endothelium, and also in the phagocytosis of complement coated particles.

Immunogen: Synthetic peptide within Human CD11b 1103-1152 / 1152.

Positive control: TF-1 cell lysate, THP-1 cell lysate, U-937 cell lysate, U937, THP-1, TF-1, human tonsil tissue, human lymph nodes tissue, human spleen tissue, human gastric cancer.

Subcellular location: Cell membrane, Membrane raft.

Database links: SwissProt: P11215 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:800
FC	1:50-1:100
IP	Use at an assay dependent concentration.
mIHC	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

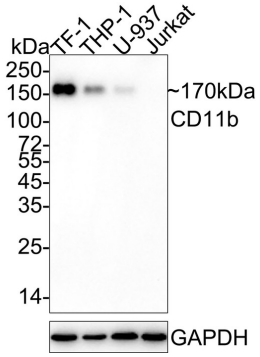

Fig1: Western blot analysis of CD11b on different lysates with Rabbit anti-CD11b antibody (ET1706-04) at 1/1,000 dilution.

Lane 1: TF-1 cell lysate (10 µg/Lane)
Lane 2: THP-1 cell lysate (15 µg/Lane)
Lane 3: U-937 cell lysate (30 µg/Lane)
Lane 4: Jurkat cell lysate (negative) (10 µg/Lane)

Predicted band size: 127 kDa
Observed band size: 170 kDa

Exposure time: 1 minute 50 seconds;

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 (ET1706-04) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

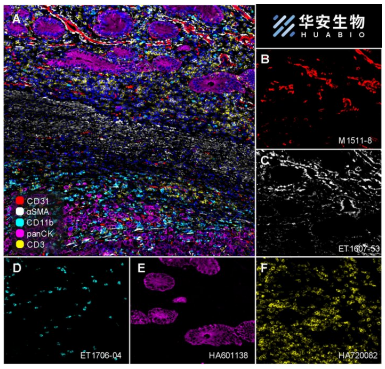

Fig2: Fluorescence multiplex immunohistochemical analysis of the human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD31 (M1511-8, red), anti-αSMA (ET1607-53, gray), anti-CD11b (ET1706-04, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human gastric cancer. Panel B: anti-CD31 stained on the endothelial cells. Panel C: anti-αSMA 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 Immuno-staining 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℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

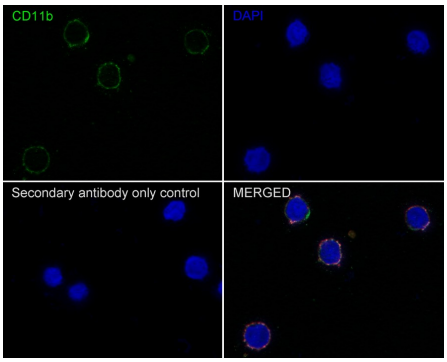

Fig3: Immunocytochemistry analysis of U937 cells labeling CD11b with Rabbit anti-CD11b antibody (ET1706-04) at 1/50 dilution.

Cells were fixed in 100% methanol for 10 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% normal goat serum for 1 hour at 37℃. Cells were then incubated with Rabbit anti-CD11b antibody (ET1706-04) 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) were used as the secondary antibody at 1/1,000 dilution.

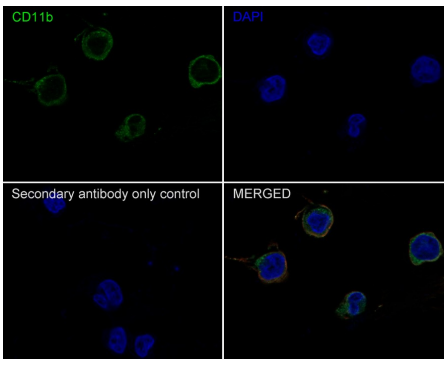

Fig4: Immunocytochemistry analysis of TF-1 cells labeling CD11b with Rabbit anti-CD11b antibody (ET1706-04) at 1/50 dilution.

Cells were fixed in 100% methanol for 10 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% normal goat serum for 1 hour at 37℃. Cells were then incubated with Rabbit anti-CD11b antibody (ET1706-04) 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.

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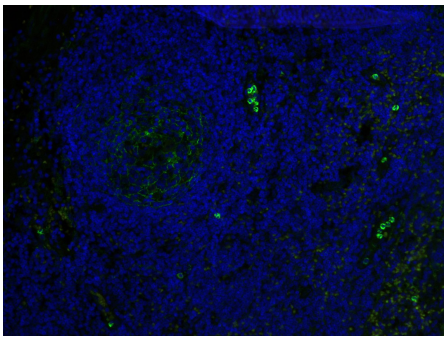

Fig5: Immunofluorescence analysis of paraffin-embedded human lymph nodes tissue labelling CD11 b (ET1706-04).

The human lymph node section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes, blocked in 10% goat serum, and then incubated with ET1706-04 at 1/50 dilution , followed by iFluorTM 488 Conjugated Goat anti-rabbit IgG at 1:1000 dilution. Nuclear was stained with Hoechst 33258 at 1/5,000 dilution.
Confocal images shows specific membrane staining of CD11b in human lymph node.

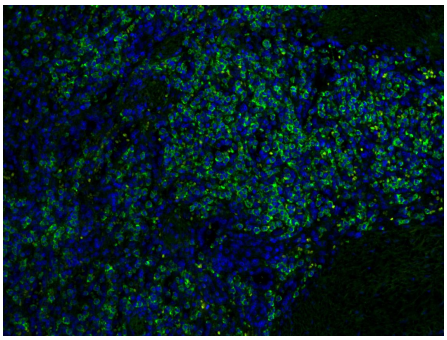

Fig6: Immunofluorescence analysis of paraffin-embedded human spleen tissue labelling CD11 b (ET1706-04).

The human spleen section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes, blocked in 10% goat serum, and then incubated with ET1706-04 at 1/50 dilution , followed by iFluorTM 488 Conjugated Goat anti-rabbit IgG at 1:1000 dilution. Nuclear was stained with Hoechst 33258 at 1/5,000 dilution.
Confocal images shows specific membrane staining of CD11b in human spleen.

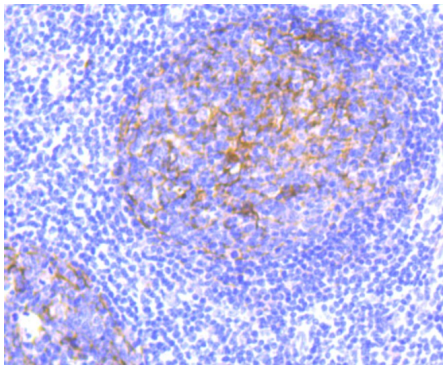

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD11b antibody (ET1706-04) at 1/50 dilution.

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 (ET1706-04) at 1/50 dilution for 0.5 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.

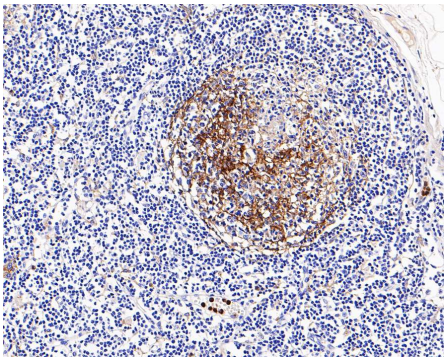

Fig8: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD11b antibody (ET1706-04) at 1/200 dilution.

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 (ET1706-04) 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.

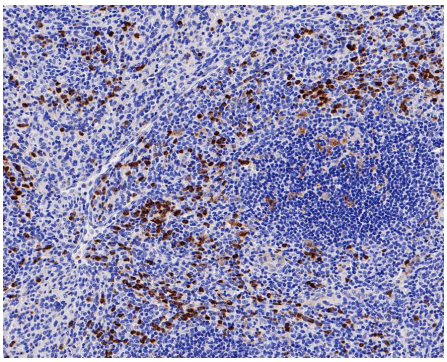

Fig9: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD11b antibody (ET1706-04) at 1/800 dilution.

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 (ET1706-04) 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.

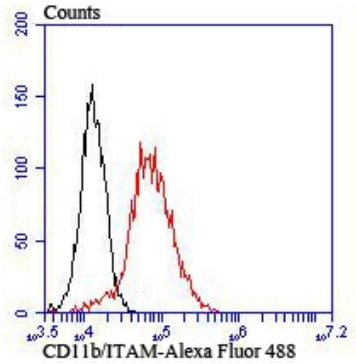

Fig10: Flow cytometric analysis of CD11b was done on THP-1 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1706-04, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

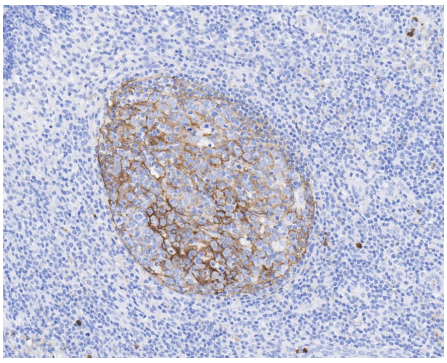

Fig11: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD11b antibody (ET1706-04) at 1/1,000 dilution.

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 (ET1706-04) 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.

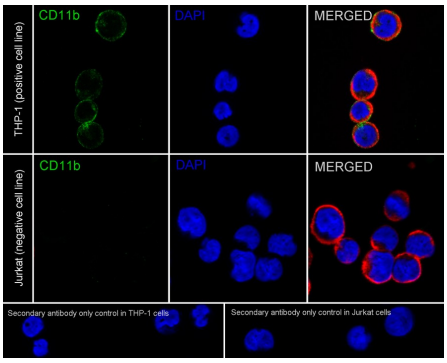

Fig12: Immunocytochemistry analysis of THP-1(+) Jurkat(-) cells labeling CD11b with Rabbit anti-CD11b antibody (ET1706-04) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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-CD11b antibody (ET1706-04) 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.

Background References

1. Yu F et al. Repetitive Model of Mild Traumatic Brain Injury Produces Cortical Abnormalities Detectable by Magnetic Resonance Diffusion Imaging, Histopathology, and Behavior. J Neurotrauma 34:1364-1381 (2017).
2. Surolia R et al. 3D pulmospheres serve as a personalized and predictive multicellular model for assessment of antifibrotic drugs. JCI Insight 2:e91377 (2017).

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