

Anti-CD34 Antibody [SI16-01]

ET1606-11



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, mIHC, FC
Molecular Wt:	Predicted band size: 41 kDa
Clone number:	SI16-01

Description: CD34 is a heavily glycosylated, transmembrane glycoprotein that is expressed on the surface of lymphohematopoietic stem and progenitor cells, small-vessel endothelial cells, embryonic fibroblasts and some cells in fetal and adult nervous tissue. CD34 antigen expression is highest in the most primitive stem cells and is gradually lost as lineage committed progenitors differentiate. The CD34 antigen is also present on capillary endothelial cells and on bone marrow stromal cells. The CD34 cytoplasmic domain has an intracellular domain that contains consensus sites for activated protein kinase C (PKC) phosphorylation as well as serine, threonine and tyrosine phosphorylation consensus sites.

Immunogen: Synthetic peptide within Human CD34 aa 336-385 / 385.

Positive control: Human non-small cell lung cancer, TF-1 cell lysate, TF-1, human tonsil tissue, human kidney tissue, mouse kidney tissue, rat kidney tissue, rat cerebellum tissue, rat cerebral cortex tissue, rat hippocampus tissue, mouse cerebellum tissue, mouse cerebral cortex tissue, mouse hippocampus tissue.

Subcellular location: Membrane.

Database links: SwissProt: P28906 Human | Q64314 Mouse
Unigene: 219720 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:100
IF-Tissue	1:50-1:100
IHC-P	1:400-1:10,000
IP	1-2µg/sample
mIHC	1:1,000-1:2,000
FC	1:1,000

Storage Buffer: 1*TBS (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 or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

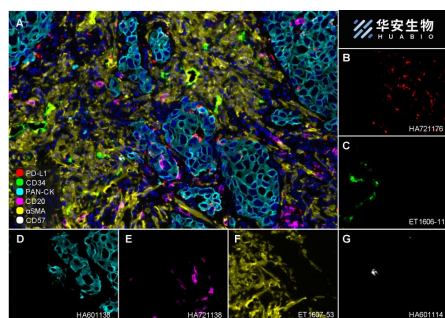


Fig1: Fluorescence multiplex immunohistochemical analysis of Human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-PD-L1 (HA721176, red), anti-CD34 (ET1606-11, green), anti-Pan-CK (HA601138, cyan), anti-CD20 (HA721138, magenta), anti-αSMA (ET1607-53, yellow) and anti-CD57 (HA601114, white) on NSCLC. Panel B: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel C: anti-CD34 stained on endothelial cells. Panel D: anti-Pan-CK stained on cancer cells. Panel E: CD20 stained on B cells. Panel F: anti-αSMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel G: anti-CD57 stained on NK cells and 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 six rounds of staining: in the order of HA721176 (1/1,000 dilution), ET1606-11 (1/1,000 dilution), HA601138 (1/3,000 dilution), HA721138 (1/2,000 dilution), ET1607-53 (1/3,000 dilution) and HA601114 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

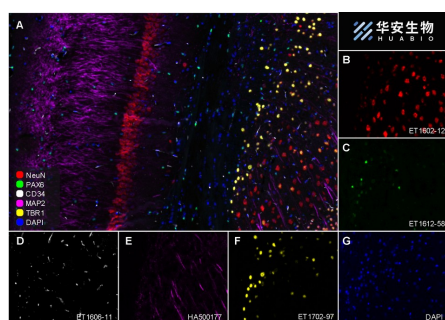


Fig2: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-PAX6 (ET1612-58, green), anti-CD34 (ET1606-11, gray), anti-MAP2 (HA500177, magenta) and anti-TBR1 (ET1702-97, yellow) on mouse brain. 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 ET1602-12 (1/5,000 dilution), ET1612-58 (1/1,000 dilution), ET1606-11 (1/2,000 dilution), HA500177 (1/5,000 dilution) and ET1702-97 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

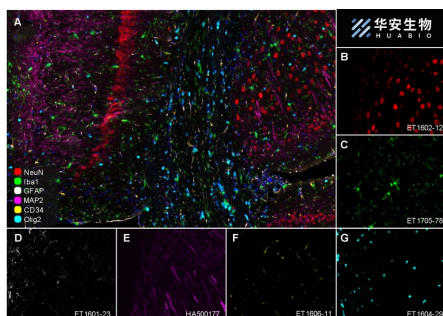
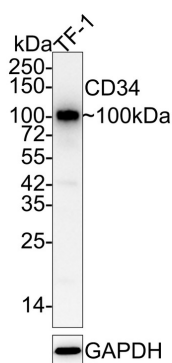


Fig3: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-Iba1 (ET1705-78, green), anti-GFAP (ET1601-23, gray), anti-Olig2 (ET1604-29, cyan), anti-MAP2 (HA500177, magenta) and anti-CD34 (ET1606-11, yellow) on mouse brain. 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 six rounds of staining: in the order of ET1602-12(1/5,000 dilution), ET1705-78 (1/2,000 dilution), ET1601-23 (1/5,000 dilution), ET1604-29 (1/1,000 dilution), HA500177 (1/5,000 dilution) and ET1606-11 (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.

Fig4: Western blot analysis of CD34 on TF-1 cell lysates with Rabbit anti-CD34 antibody (ET1606-11) at 1/2,000 dilution.



Lysates/proteins at 10 µg/Lane.

Predicted band size: 41 kDa

Observed band size: 100 kDa

Exposure time: 5 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 (ET1606-11) at 1/2,000 dilution was used in 5% NFDm/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

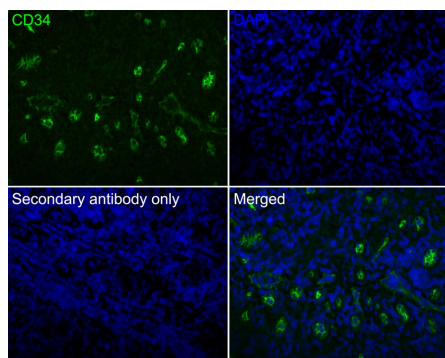


Fig5: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling CD34 with Rabbit anti-CD34 antibody (ET1606-11) at 1/50 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1606-11, green) at 1/50 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).

Hangzhou Huaan Biotechnology Co., Ltd.

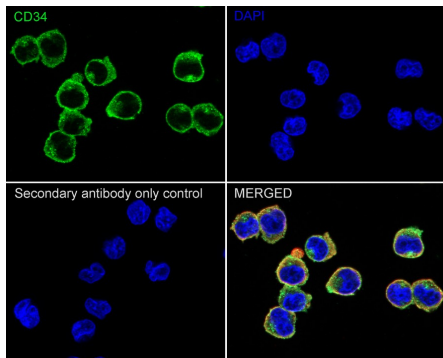
Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

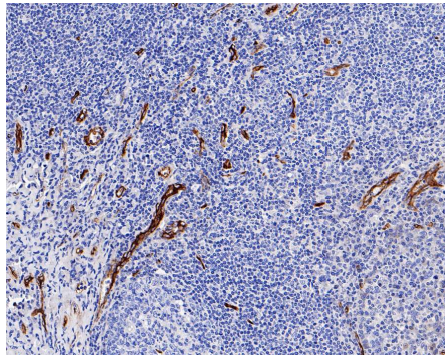
Fig6: Immunocytochemistry analysis of TF-1 cells labeling CD34 with Rabbit anti-CD34 antibody (ET1606-11) at 1/100 dilution.



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-CD34 antibody (ET1606-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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.

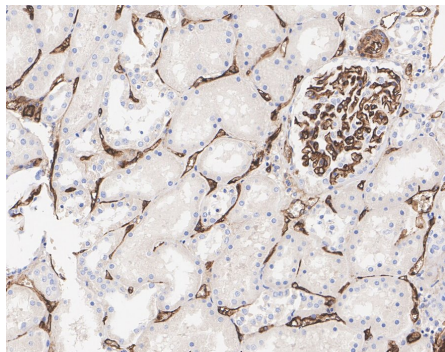
Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD34 antibody (ET1606-11) 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 (ET1606-11) 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.

Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-CD34 antibody (ET1606-11) 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 (ET1606-11) 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.

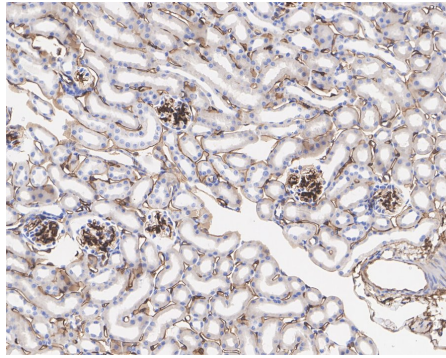


Fig9: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/1,000 dilution.

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 (ET1606-11) 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.

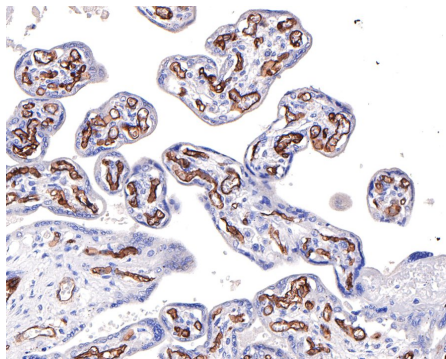


Fig10: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/400 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1606-11) 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.

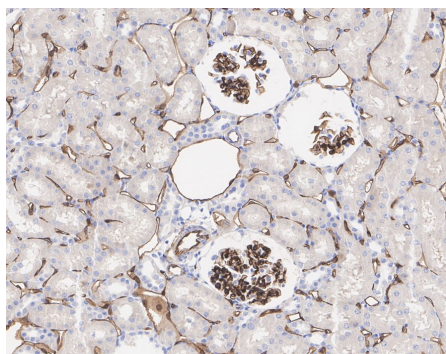


Fig11: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/1,000 dilution.

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 (ET1606-11) 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.

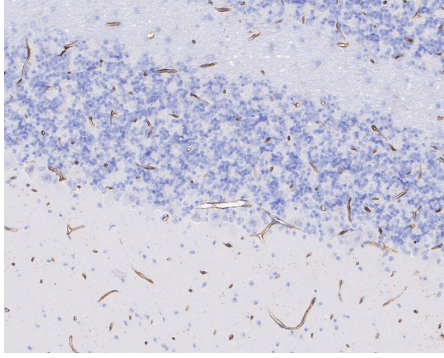


Fig12: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

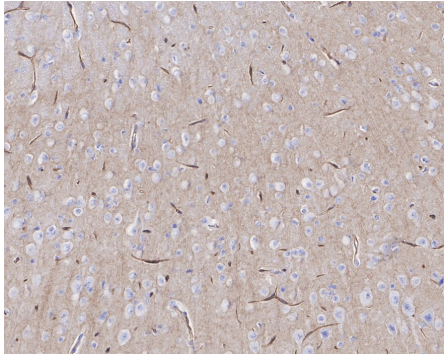


Fig13: Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

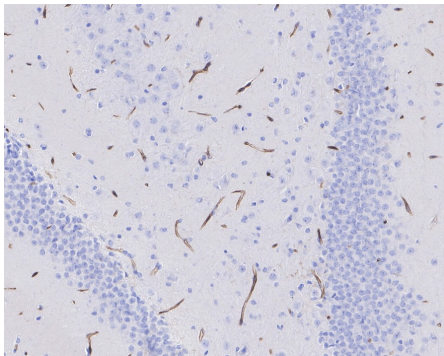


Fig14: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

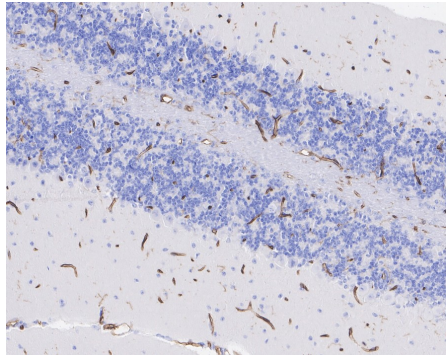


Fig15: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

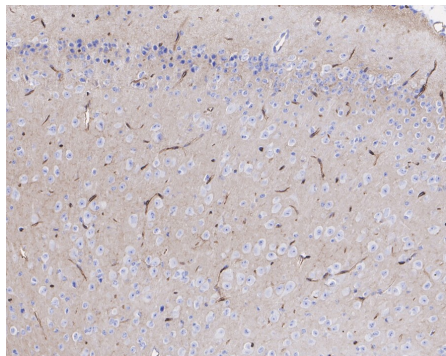


Fig16: Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

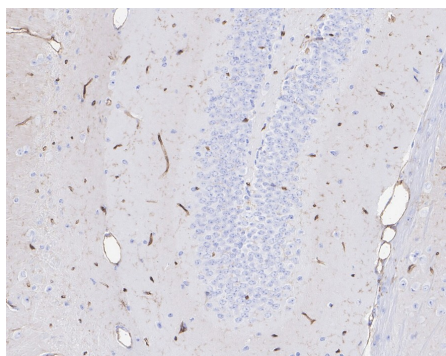


Fig17: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-CD34 antibody (ET1606-11) at 1/10,000 dilution.

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 (ET1606-11) at 1/10,000 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.

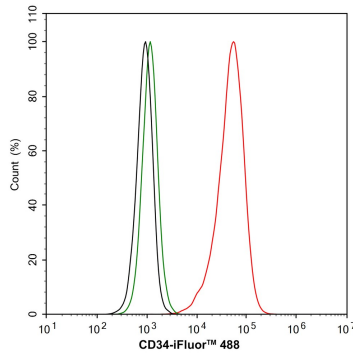


Fig18: Flow cytometric analysis of TF-1 cells labeling CD34.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1606-11, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

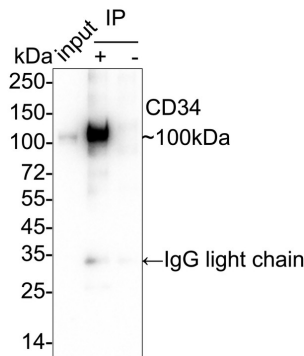


Fig19: CD34 was immunoprecipitated from 0.2 mg TF-1 cell lysate with ET1606-11 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1606-11 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: TF-1 cell lysate (input)

Lane 2: ET1606-11 IP in TF-1 cell lysate

Lane 3: Rabbit IgG instead of ET1606-11 in TF-1 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 6 seconds; ECL: K1801

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

Background References

1. Lin, SZ. et al. 2015. Emodin inhibits angiogenesis in pancreatic cancer by regulating the transforming growth factor-β/drosophila mothers against decapentaplegic pathway and angiogenesis-associated microRNAs. *Molecular medicine reports*. 12: 5865-71.
2. Corradi, LS. et al. 2013. Structural and ultrastructural evidence for telocytes in prostate stroma. *J. Cell. Mol. Med.* 17: 398-406.

Hangzhou Huan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn