Anti-CXCR5 Antibody [JB11-40]

ET7107-74

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JB11-40

Description: Burkitt's lymphoma receptor 1 (Blr1) is a lymphocyte specific chemokine receptor expressed at low levels in secondary lymphoid tissues and in defined structures of the cerebellum. The G-protein coupled receptor has significant homology to other chemokine receptors. Stimulation of Blr1 by its ligand, B-lymphocyte chemo-attractant (BLC) results in an influx of calcium into the cell and the chemotaxis of the cell. Blr1 is required for B-cell migration into splenic and Peyer's patch follicles. BLC expression in Peyer's patches is highest in germinal centers, where B cells undergo somatic mutation and affinity maturation.

Immunogen: Synthetic peptide within C-terminal human CXCR5.

Positive control: Daudi cell lysate, EL4 cell lysate, Neuro-2a cell lysate, C6 cell lysate, mouse spleen tissue lysate, rat spleen tissue lysate, Daudi, Neuro-2a, rat kidney tissue, human tonsil tissue, mouse heart tissue

Subcellular location: Cell membrane.

Database links: SwissProt: P32302 Human | Q04683 Mouse | P34997 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:50-1:200
FC	1:50-1:100
IF-Cell	1:250

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

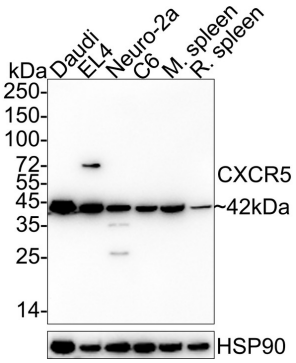

Fig1: Western blot analysis of CXCR5 on different lysates with Rabbit anti-CXCR5 antibody (ET7107-74) at 1/2,000 dilution.

Lane 1: Daudi cell lysate
Lane 2: EL4 cell lysate
Lane 3: Neuro-2a cell lysate
Lane 4: C6 cell lysate
Lane 5: Mouse spleen tissue lysate
Lane 6: Rat spleen tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 1 minute 34 seconds; ECL: K1801;

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 (ET7107-74) at 1/2,000 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.

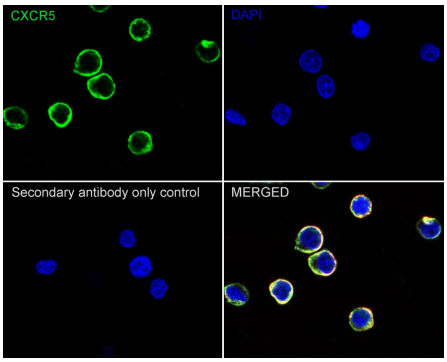

Fig2: Immunocytochemistry analysis of Daudi cells labeling CXCR5 with Rabbit anti-CXCR5 antibody (ET7107-74) at 1/250 dilution.

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-CXCR5 antibody (ET7107-74) at 1/250 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.

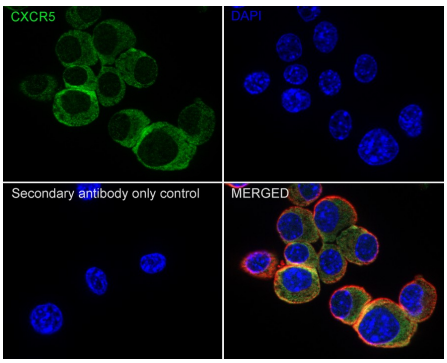

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling CXCR5 with Rabbit anti-CXCR5 antibody (ET7107-74) at 1/250 dilution.

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-CXCR5 antibody (ET7107-74) at 1/250 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.

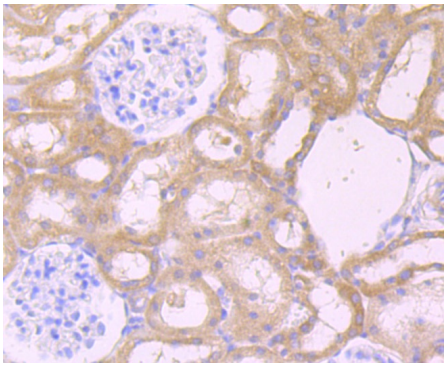

Fig4: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-CXCR5 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-74, 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.

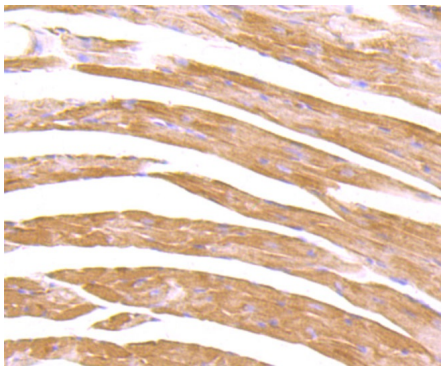

Fig5: Immunohistochemical analysis of paraffin-embedded mouse heart tissue using anti-CXCR5 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-74, 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.

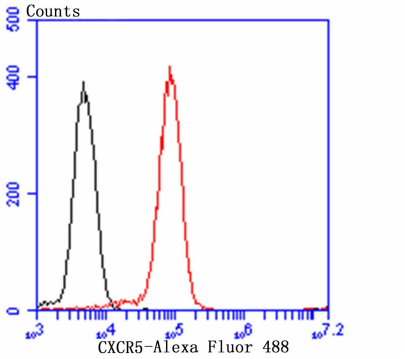

Fig6: Flow cytometric analysis of CXCR5 was done on Daudi cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7107-74, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Sjoeblom T et al. The consensus coding sequences of human breast and colorectal cancers. Science 314:268-274 (2006).
2. Barella L et al. Sequence variation of a novel heptahelical leucocyte receptor through alternative transcript formation. Biochem J 309:773-779 (1995).