Anti-CCR7 Antibody [SR36-04]

ET1602-22

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 43 kDa
Clone number:	SR36-04

Description: C-C chemokine receptor type 7 is a protein that in humans is encoded by the CCR7 gene. Two ligands have been identified for this receptor: the chemokines (C-C motif) ligand 19 (CCL19/ELC) and (C-C motif) ligand 21 (CCL21). CCR7 has also recently been designated CD197 (cluster of differentiation 197). The protein encoded by this gene is a member of the G protein-coupled receptor family. This receptor was identified as a gene induced by the Epstein-Barr virus (EBV), and is thought to be a mediator of EBV effects on B lymphocytes. This receptor is expressed in various lymphoid tissues and activates B and T lymphocytes. CCR7 has been shown to stimulate dendritic cell maturation. CCR7 is also involved in homing of T cells to various secondary lymphoid organs such as lymph nodes and the spleen as well as trafficking of T cells within the spleen. Activation of Dendritic cells in peripheral tissues induces CCR7 expression on the cell's surface, which recognize CCL19 and CCL21 produced in the Lymph node and increases dendritic cell expression of co-stimulation molecules (B7), and MHC class I or MHC class II.

Immunogen: Synthetic peptide within Human CCR7 aa 13-62 / 378.

Positive control: HDLM-2 cell lysate, Jurkat cell lysate, Daudi cell lysate, MCF7 cell lysate, mouse spleen tissue lysate, mouse pancreas tissue lysate, rat spleen tissue lysate, Raji cell lysate, EL4 cell lysate, RAW264.7 cell lysate, C6 cell lysate, Daudi, RAW264.7, C6, human tonsil tissue, human spleen tissue.

Subcellular location: Cell membrane.

Database links: SwissProt: P32248 Human | P47774 Mouse
Unigene: 229736 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:200-1:500
IF-Tissue	1:200
IHC-P	1:200
IP	1-2μg/sample

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

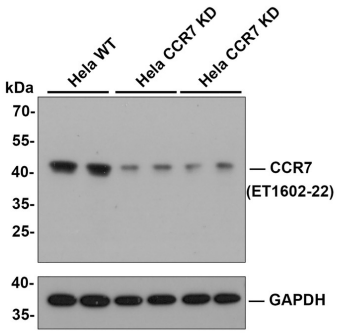

Fig1: All lanes: Western blot analysis of CCR7 with anti-CCR7 antibody (ET1602-22) at 1:5,000 dilution.
Lane 1/2: Wild-type Hela whole cell lysate (10 µg).
Lane 3/4: CCR7 fragment 1 knockdown Hela whole cell lysate (10 µg).
Lane 5/6: CCR7 fragment 2 knockdown Hela whole cell lysate (10 µg).

ET1602-22 was shown to specifically react with CCR7 in wild-type Hela cells. Weakened was observed when CCR7 knockdown sample was tested. Wild-type and CCR7 knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1602-22, 1/500) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) was used in 5% BSA 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.

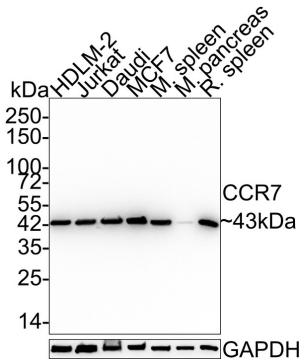

Fig2: Western blot analysis of CCR7 on different lysates with Rabbit anti-CCR7 antibody (ET1602-22) at 1/5,000 dilution.

Lane 1: HDLM-2 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: Daudi cell lysate
Lane 4: MCF7 cell lysate
Lane 5: Mouse spleen tissue lysate
Lane 6: Mouse pancreas tissue lysate (low expression)
Lane 7: Rat spleen tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 24 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 (ET1602-22) at 1/5,000 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.

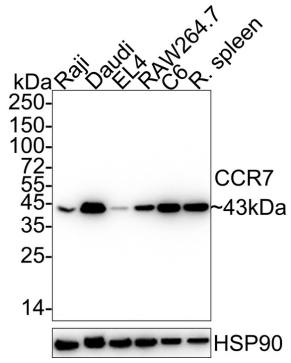

Fig3: Western blot analysis of CCR7 on different lysates with Rabbit anti-CCR7 antibody (ET1602-22) at 1/2,000 dilution.

Lane 1: Raji cell lysate
Lane 2: Daudi cell lysate
Lane 3: EL4 cell lysate
Lane 4: RAW264.7 cell lysate
Lane 5: C6 cell lysate
Lane 6: Rat spleen tissue lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 10 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 (ET1602-22) 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.

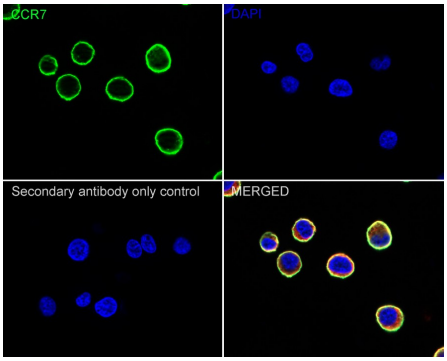

Fig4: Immunocytochemistry analysis of Daudi cells labeling CCR7 with Rabbit anti-CCR7 antibody (ET1602-22) at 1/250 dilution.

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-CCR7 antibody (ET1602-22) 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.

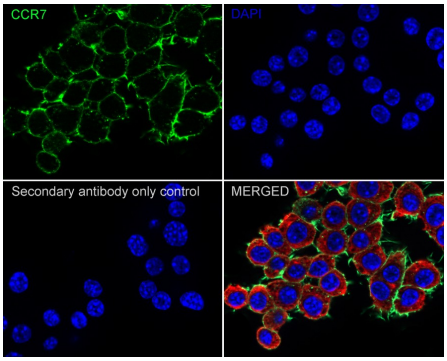

Fig5: Immunocytochemistry analysis of RAW264.7 cells labeling CCR7 with Rabbit anti-CCR7 antibody (ET1602-22) at 1/500 dilution.

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-CCR7 antibody (ET1602-22) at 1/500 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.

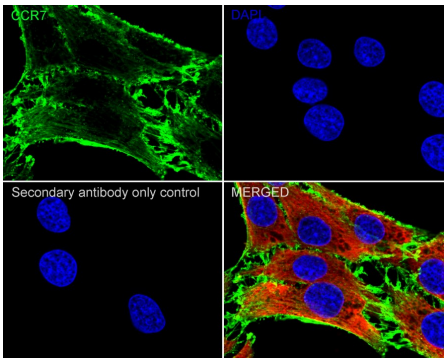

Fig6: Immunocytochemistry analysis of C6 cells labeling CCR7 with Rabbit anti-CCR7 antibody (ET1602-22) at 1/200 dilution.

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-CCR7 antibody (ET1602-22) at 1/200 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.

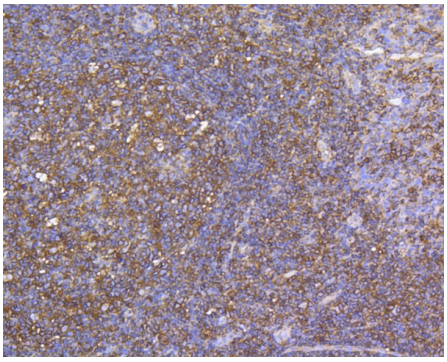

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-CCR7 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-22, 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.

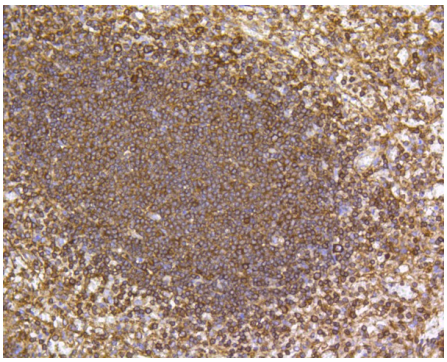

Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-CCR7 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-22, 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.

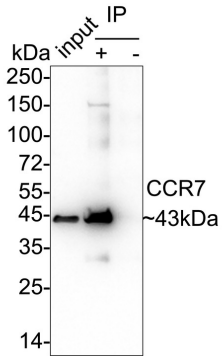

Fig9: CCR7 was immunoprecipitated from 0.2 mg Daudi cell lysate with ET1602-22 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1602-22 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Daudi cell lysate (input)
Lane 2: ET1602-22 IP in Daudi cell lysate
Lane 3: Rabbit IgG instead of ET1602-22 in Daudi cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 8 seconds; ECL: K1802

Background References

1. Pang MF et al. TGF-β1-induced EMT promotes targeted migration of breast cancer cells through the lymphatic system by the activation of CCR7/CCL21-mediated chemotaxis. Oncogene N/A:N/A (2015).
2. Guo J et al. Effect of CCR7, CXCR4 and VEGF-C on the lymph node metastasis of human pancreatic ductal adenocarcinoma. Oncol Lett 5:1572-1578 (2013).