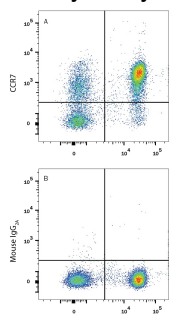
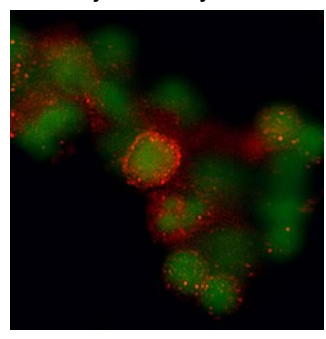
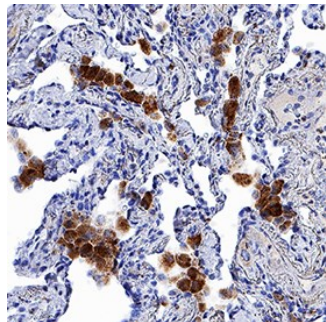


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human CCR7.
Source	Monoclonal Mouse IgG _{2A} Clone # 150503
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human CCR7 transfectants Met1-Pro378 Accession # AAA58615
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS		
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.		
	Recommended Concentration	Sample
Dual RNAscope ISH-IHC Compatible	3-25 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
CyTOF-reported	Mei, H.E. <i>et al.</i> (2015) J. Immunol. 194 : 2022. Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize CCL19/MIP-3β-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CCR7. The Neutralization Dose (ND ₅₀) is typically 1-5 µg/mL in the presence of 50 ng/mL Recombinant Human CCL19/MIP-3β.	

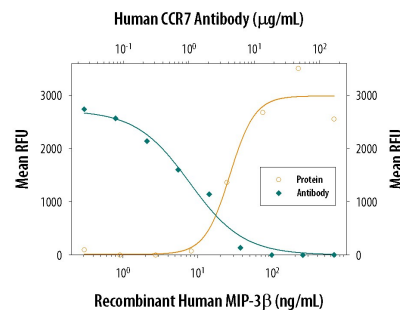
DATA	
<p>Flow Cytometry</p>  <p>Detection of CCR7 in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with Mouse Anti-Human CD4 PE-conjugated Monoclonal Antibody (Catalog # FAB3791P) and either (A) Mouse Anti-Human CCR7 Monoclonal Antibody (Catalog # MAB197) or (B) Mouse IgG_{2A} Isotype Control (Catalog # MAB003) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). View our protocol for Staining Membrane-associated Proteins.</p>	<p>Immunocytochemistry</p>  <p>CCR7 in Human PBMCs. CCR7 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using 25 µg/mL Mouse Anti-Human CCR7 Monoclonal Antibody (Catalog # MAB197) for 3 hours at room temperature. Cells were stained (red) and counterstained (green). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>

Immunohistochemistry



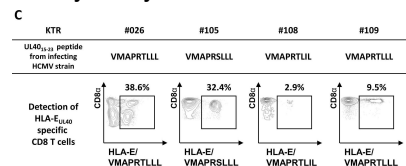
CCR7 in Human Lung. CCR7 was detected in immersion fixed paraffin-embedded sections of human lung using Mouse Anti-Human CCR7 Monoclonal Antibody (Catalog # MAB197) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in macrophages. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Neutralization



Chemotaxis Induced by CCL19/MIP-3β and Neutralization by Human CCR7 Antibody. Recombinant Human CCL19/MIP-3β (Catalog # Catalog # 361-MI) chemoattracts the BaF3 mouse pro-B cell line transfected with human CCR7 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # Catalog # AR002). Chemotaxis elicited by Recombinant Human CCL19/MIP-3β (50 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human CCR7 Monoclonal Antibody (Catalog # MAB197). The ND₅₀ is typically 1-5 µg/mL.

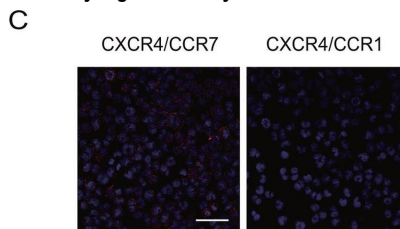
Flow Cytometry



Detection of Human CCR7 by Flow Cytometry

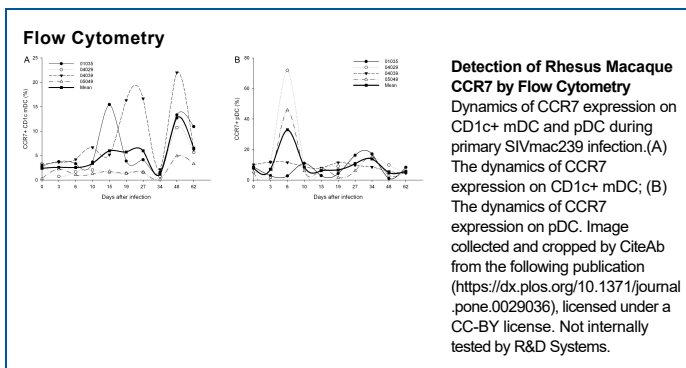
HCMV strain-dependent variability of UL4015-23 sequences and HCMV strain-specific HLA-EUL40 T-cell response in hosts. (A, B) Genomic DNAs isolated from HCMV positive blood samples in HCMV+ transplant recipients (n = 25) were sequenced for the identification of UL40 protein (amino acids 1–221) provided by the circulating HCMV strains. (A) Amino acid variability, expressed as a number of amino acid variants, within the HLA-E-binding peptide (UL4015-23, shown in red) among the sequence for HCMV UL40 signal peptide (UL401-37, shown in grey). A total of 32 UL40 sequences from 25 hosts were analysed. UL40 protein sequence from the Merlin HCMV clinical strain was used as reference. Positions 1 to 9 of residues in the HLA-E-binding peptide (UL4015-23) are indicated. (B) Sequence LOGO of the UL4015-23 HLA-E-binding peptide from 25 transplanted hosts. The height of the letter is proportional to the frequency of each amino acid in a given position (P1 to P9). Major anchor residues for binding in the HLA-E peptide groove are indicated in blue. Red letters highlight the important variability observed in position 8 of the HLA-E-binding peptide. Grey boxes correspond to a constitutive deletion of the corresponding amino acid in the UL40 sequence from the infecting viral strain. (C) Representative dot plot analyses showing the detection of strain-specific anti-UL40 HLA-E-restricted CD8 T-cell responses in 4 KTRs (KTR#026, #105, #108 and #109). Frequencies (%) of the HLA-EUL40-specific T cells among total circulating αβ CD8 T cells are indicated. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.ppat.1007041>), licensed under a CC-BY license. Not internally tested by R&D Systems.

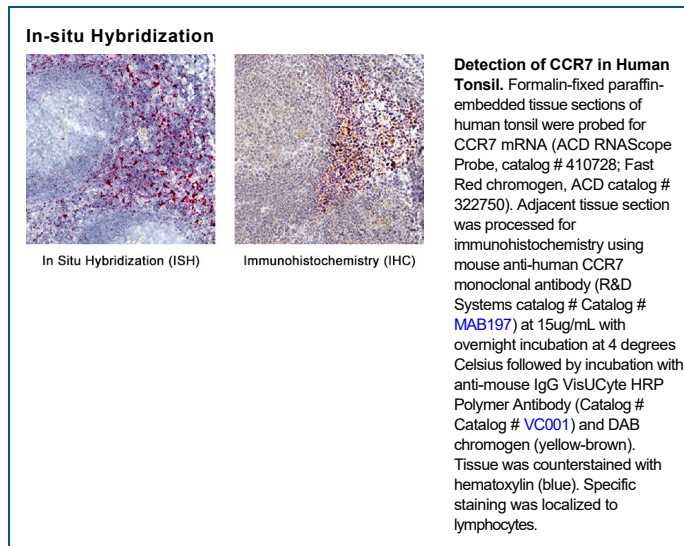
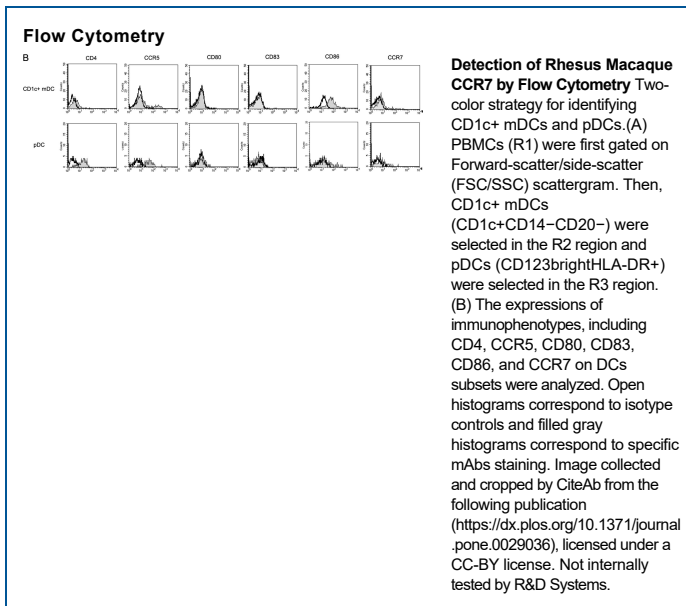
Proximity Ligation Assay



Detection of Human CCR7 by Proximity Ligation Assay

CXCR4 ligand binding facilitates CXCR4/CCR7 hetero-oligomer formation. (A) CCR7 homo-oligomer formation in H9 cells after treatment with or without 1 μg/ml recombinant gp120 was examined by in situ PLA. The number of in situ PLA signals per cell was counted by using the Duolink Image Tool software. The result shown is a representative result from three independent experiments showing the mean number of the signals plotted on the vertical axis. *, p < 0.05 by Mann-Whitney's U test. (B) CCR7 homo-oligomer formation in H9 cells after treatment with 100 ng/ml CXCL12 was examined, as described in (A). Quantification of CCR7 homo-oligomers after CXCL12 pretreatment in the presence or absence of 1 or 5 μg/ml AMD3100. The result shown is a representative result from five independent experiments. *, p < 0.05 by Mann-Whitney's U test. (C) CXCR4/CCR7 or CXCR4/CCR1 hetero-oligomer formation in H9 cells by in situ PLA using the indicated combinations of antibodies. The z-stack images derived from sections covering 10 μm with a 0.5-μm step are shown. Scale bar represents 50 μm. (D) Quantification of the detected in situ PLA signals per cell was performed. The result shown is a representative result from three independent experiments. *, p < 0.05 by Mann-Whitney's U test. (E) CXCR4/CCR7 hetero-oligomer formation in H9 cells after treatment with 1 μg/ml gp120 in a native form (N) or control heat-denatured form (D) was examined by the PLA using the indicated combinations of antibodies. (F) CXCR4/CCR7 hetero-oligomer formation was examined with 100 ng/ml CXCL12 as described in (E). Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0117454>), licensed under a CC-BY license. Not internally tested by R&D Systems.





PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

CCR7 (Chemokine Receptor 7; also CD197) is a 7 transmembrane (7TM) G protein-coupled chemokine receptor for the homeostatic chemokines CCL19/MIP-3 beta and CCL21/6CKine. CCL19 and CCL21 are constitutively expressed by high endothelial venule epithelial cells or fibroblastic reticular cells in secondary lymphoid organs. CCR7 is upregulated on dendritic cells, naïve and memory T cells, Treg, NK cells, and B cells following inflammatory stimulation. Its expression enables the function of immune cell trafficking to and retention in regional lymph nodes for expansion of the adaptive immune response. Human CCR7 shares 87% amino acid sequence identity with mouse CCR7.