CD197 (CCR7) Monoclonal Antibody (4B12), Functional Grade, eBioscience™

Product Details	
Size	500 µg
Species reactivity	Mouse
Published species	Human, Mouse
Host / Isotype	Rat / IgG2a, kappa
Class	Monoclonal
Type	Antibody
Clone	4B12
Conjugate	Functional Grade
Form	Liquid
Concentration	1 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2
Contains	no preservative
Storage conditions	4° C
RRID	AB_494123

Applications	Tested Dilution	Publications
Control (Ctrl)	Assay-Dependent	-
Flow Cytometry (Flow)	1 µg/test	20 Publications
Functional assay (FN)	Assay-Dependent	1 Publication
Immunoprecipitation (IP)	Assay-Dependent	-
Immunocytochemistry (ICC/IF)	-	1 Publication
Immunohistochemistry (IHC)	-	1 Publication
Inhibition Assays (IA)	-	1 Publication

Product Specific Information

Description: The 4B12 monoclonal antibody reacts with mouse CCR7, also known as EBI-1 and CD197. CCR7 is a chemokine receptor for the chemokines CCL19 (CKβ11, ELC, MIP3β, Scya19, Exodus-3) and CCL21 (CKβ9, SLC, MIP2β, Scya21, Exodus-2). In recent years, the role of chemokines in directing the migration of lymphocytes has been well-characterized. One of the most important mediators of homeostatic trafficking of naive T cells to secondary lymphoid organs (SLO) is the chemokine receptor CCR7. Binding of its ligands, CCL19 and CCL21, mediates the trans endothelial migration of T cells across high endothelial venules into SLO. It has also been demonstrated that CCR7 plays a role in the localization of dendritic cells and B cells during an immune response.

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In addition to its significant role in the chemotaxis of lymphocytes, human CCR7 has also been recognized as a marker for a distinct subset of memory T cells, the central memory (TCM) population. These cells are characterized by the expression of CCR7 and CD62L and reside within peripheral lymphoid organs. CCR7 also plays a role in thymocyte development and its deficiency leads to disturbed thymic architecture, aberrant T cell development, and limited thymocyte expansion.

For optimal visualization of CCR7 expression on different cell types it is necessary to use multi-color staining to discriminate different cell subsets as well as following the protocol (incubation at 37C may be necessary). To address specificity, the staining profile of 4B12 has been compared to a polyclonal antibody generated against a CCR7 peptide (Bjorkdahl et al). This analysis confirms that the polyclonal antibody and 4B12 stain similar populations of cells. Furthermore, 4B12 stains mouse CCR7-GFP fusion protein-transfected RBL cells (see data in cat. 14-1971).

Applications Reported: This 4B12 antibody has been reported for use in flow cytometric analysis, and immunoprecipitation.

Applications Tested: The 4B12 antibody has been tested by flow cytometric analysis of mouse splenocytes and thymocytes. This can be used at less than or equal to 1 µg per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

Important: Staining with the 4B12 monoclonal antibody requires different conditions than typically used for surface-antigen staining. Please use the protocol below. Moreover, we have found that staining at 37°C, rather than 2-8°C, results in brighter 4B12 staining, as well as better resolution between positive and negative populations. Please see data for the PE 4B12 (cat. 12-1971) which demonstrates a comparison of staining at 2-8°C and 37°C. Staining with 4B12 at 37°C is not expected to interfere with co-staining other antigens, however this should be evaluated for individual experiments.

1. Prepare cell suspension as normal and block Fc gammaIIR/Fc gammaIIR with 5 µg/million cells purified anti-mouse CD16/32 (cat. 14-0161) for 15 minutes on ice. If red blood cell lysis is carried out as part of cell preparation, ensure that fixatives are not present in the red blood cell lysis solution as this will eliminate 4B12 staining.

2. Without washing, add 1 µg/million cells 4B12 and incubate in a 37°C waterbath or at 2-8°C (please see notes above) for 0.5 hours.

3. Wash cells 1X with 3 mL of Flow Cytometry Staining Buffer (cat. 00-4222) and decant supernatant.

4. Add secondary antibody and incubate at 2-8°C for 30 minutes.

5. Wash cells 1X with 3 mL of Flow Cytometry Staining Buffer (cat. 00-4222) and decant supernatant.Analyze cells on flow cytometer or proceed with secondary staining on ice as normal.

Note: Co-staining mouse CCR7 with the 4B12 antibody and the CCR7 ligand CCL19-Fc (cat. 14-1972) may be difficult due to different binding conditions required for the antibody versus the ligand, and steric hindrance which may prevent co-staining of 4B12 and CCL19-Fc. Cross-blocking experiments have demonstrated that 4B12 binding is able to prevent the detectable binding of CCL19-Fc, however not the opposite. Furthermore, the correlation between 4B12 and CCL19-Fc staining may be difficult to predict due to the presence of unknown CCL19-Fc receptors in addition to CCR7.

Storage and handling: Use in a sterile environment.

Filtration: 0.2 µm post-manufacturing filtered.

Purity: Greater than 90%, as determined by SDS-PAGE.

Endotoxin Level: Less than 0.001 ng/µg antibody, as determined by LAL assay.

Aggregation: Less than 10%, as determined by HPLC.

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