Anti-Human CD185 (J252D4)

	REF	Σ			
APC	185A-100T	100 test	5 µL/test	0,05 mg/ml	RUO

1. PRODUCT DESCRIPTION

Clone: J252D4;
Isotype: Mouse IgG1, κ;
Tested application: flow cytometry (Quality tested);
Immunogen: Human CXCR5-transfected cells;
Species reactivity: Human;
Storage instruction: store in the dark at 2-8 °C;
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN_3);
Recommended usage: Immunostep's anti-human CD185, clone J252D4, is a monoclonal antibody intended for the identification of cells expressing CXCR5 protein in peripheral blood using a compatible flow cytometer. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10^6 cells or 100 µl of sample;
Presentation: liquid;
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
Purification: Affinity chromatography;
Other names: CXCR5, BLR1, MDR15;
Gene ID: 643.

2. ANTIGEN DETAILS

Large description: CD185, a 42 kD G-protein coupled receptor with seven transmembrane regions. CXCR5 is expressed by mature B cells, follicular helper T cells, Burkitt's lymphoma cells, and a subset of neurons. It plays a crucial role in directing cell migration to the B cell follicles within secondary lymphoid organs, which are key sites for initiating immune responses.

The ligand for the CXCR5 chemokine receptor is CXCL13 (also known as B-lymphocyte chemoattractant or BLC). CXCR5 plays an important role in the positioning and cognate interactions of B-cell chronic lymphocytic leukemia (CLL) cells with CXCL13-secreting CD68+ accessory cells within the lymphoid tissues. This interaction helps support the survival and proliferation of CLL cells in their protective microenvironment.[1]

The high expression of CXCR5 on T follicular helper cells (T(FH)) and a subset of central memory CD4 T cells (T(CM)), suggesting that CXCR5(+) T(CM) may function similarly to T(FH) cells in supporting humoral immune responses. CXCR5(+) T(CM) exhibit B cell helper qualities, expressing high levels of CXCL13, inducing plasma cell differentiation and Ig secretion, and showing responsiveness to ICOS ligand costimulation and IL-10 secretion. These attributes are acquired through interaction with B cells, indicating a specialized role in promoting quick and efficient secondary humoral immune responses. In conclusion, CXCR5(+) T(CM) are proposed as a distinct memory cell subset specialized in supporting antibody-mediated immune responses[2].

3. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties.

Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

4. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

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5. PROTOCOL

Direct Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100 ul (10⁶ cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

- **Indirect Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (10⁶ cells/test) of the sample to a 12 x 75 mm polystyrene test tube
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

6. REFERENCES

1. Bürkle A, Niedermeier M, Schmitt-Gräff A, Wierda WG, Keating MJ, Burger JA. Overexpression of the CXCR5 chemokine receptor, and its ligand, CXCL13 in B-cell chronic lymphocytic leukemia. Blood. 2007 Nov 1;110(9):3316-25. doi: 10.1182/blood-2007-05-089409. Epub 2007 Jul 25. PMID: 17652619.

2. Chevalier N, Jarrossay D, Ho E, Avery DT, Ma CS, Yu D, Sallusto F, Tangye SG, Mackay CR. CXCR5 expressing human central memory CD4 T cells and their relevance for humoral immune responses. J Immunol. 2011 May 15;186(10):5556-68. doi: 10.4049/jimmunol.1002828. Epub 2011 Apr 6. PMID: 21471443.

7. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

8. MANUFACTURED BY:

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