Anti-PTK7 (CCK-4) antibodies

Anti-PTK7 (CCK-4)-PE	130-091-364
Anti-PTK7 (CCK-4)-APC	130-091-366
Anti-PTK7 (CCK-4)-Biotin	130-091-365
Anti-PTK7 (CCK-4) pure	130-091-578

Antibodies

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1. Description

Clone 188B (isotype: mouse IgG2a).

Product format 1 mL Anti-PTK7 (CCK-4) antibodies, human: monoclonal anti-PTK7 (CCK-4) antibodies conjugated to R-phycoerythrin (**PE**), allophycocyanin (**APC**), biotin (**Biotin**), or as unconjugated antibody (**pure**).

Product size Conjugates: For 10^9 total cells, up to 100 stainings. Pure antibody: 100 µg/mL.

Storage Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The monoclonal antibody 188B recognizes human protein tyrosin kinase-7 (PTK7), which is also known as colon carcinoma kinase-4 (CCK-4). PTK7 (CCK-4) is a receptor protein tyrosin kinase (RPTK)-like molecule which contains a catalytically inactive tyrosin kinase domain.[1] The PTK7 (CCK-4) gene is located on chromosom 6p21.1-p12.2 and is organized onto 20 exons. PTK7 (CCK-4) mRNA is detected in normal human melanocytes, colon carcinoma cells, and lung, liver, pancreas, kidney and placenta tissue. Recently, using the specific monoclonal antibody 188B, PTK7 (CCK-4) was shown to be expressed in blood and bone marrow, on plasmacytoid dendritic cells, CD141 (BDCA-3)^{high} type-2 myeloid dendritic cells, and CD34$^+$ hematopoietic progenitor cells.[1] PTK7 (CCK-4) was detected on early (CD34$^+$ CD133$^+$) and late (CD34$^+$ CD133$^-$) hematopoietic progenitor cells. In tonsils, PTK7 (CCK-4) was also found on some T cells.
In healthy donors, PTK7 (CCK-4)$^+$ cells represent about 0.8% of human peripheral blood mononuclear cells (PBMCs), and about 13% of bone marrow mononuclear cells (BMMNCs).

Product applications

- Identification and enumeration of PTK7 (CCK-4)$^+$ cells by flow cytometry or fluorescence microscopy, or immunohistochemical staining and analysis by light microscopy.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Human PTK7 (CCK-4)$^+$ cells can be isolated by using the Anti-PTK7 (CCK-4) MicroBead Kit, human (# 130-091-367).

1.2 Examples of staining concentrations

Anti-PTK7 (CCK-4) conjugate	PE	APC	Biotin	pure
	Recommended antibody dilution			
Flow cytometry[a]				
- in general	1:11	1:11	1:11	
- formaldehyde-fixed cells[b]	1:11	1:11	1:11	
- Anti-PTK7 (CCK-4) MicroBead-labeled cells	1:11	1:11	1:11	
Immunohistochemistry[c]				

a) Given antibody dilutions are for a cell concentration of up to 1×10^8 cells/mL buffer.
b) For optimal results, cells have to be stained prior to fixation.
c) For immunohistochemical staining the optimal antibody dilution has to be tested.

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- FcR Blocking Reagent, human (# 130-059-901): Fc receptor-mediated fluorescent staining can be avoided by blocking of Fc receptor using FcR Blocking Reagent, human.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-PTK7 (CCK-4)-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

140-000-935.01

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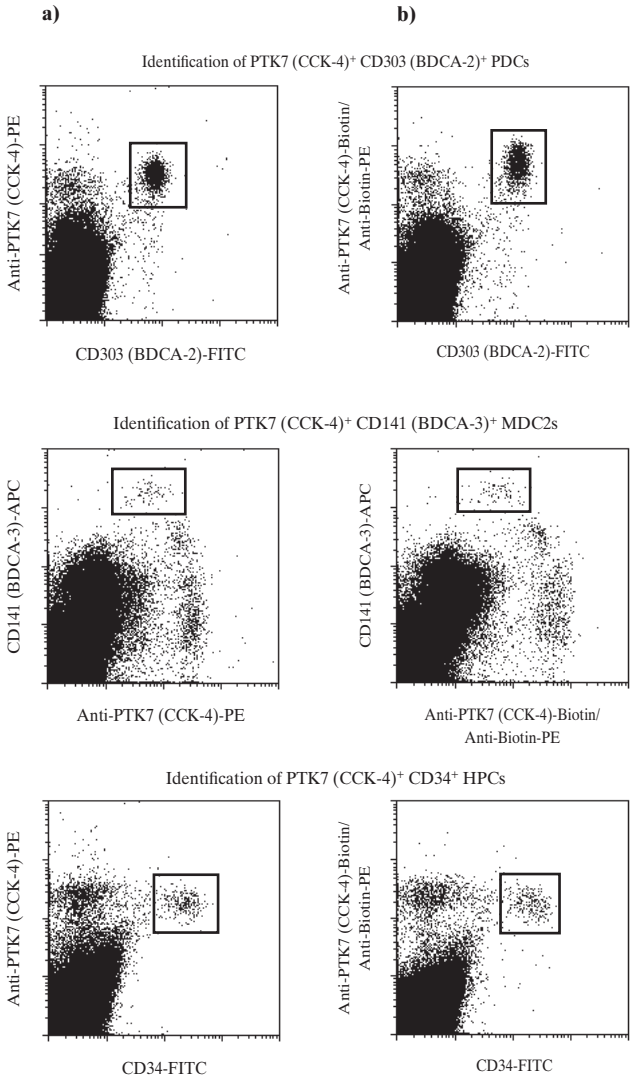
2. General protocol for immunofluorescent staining using Anti-PTK7 (CCK-4)-PE, -APC or -Biotin

▲ Volumes for fluorescent labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated (e.g. when working with standard 10^6 cells, use the same volume as indicated). When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10^7 cells in 80 µL of buffer.
2. Add 20 µL of FcR Blocking Reagent.
3. Add 10 µL of Anti-PTK7 (CCK-4) antibodies.
4. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
6. If Anti-PTK7 (CCK-4)-Biotin was used, resuspend cell pellet in 100 µL buffer, add 10 µL Anti-Biotin antibody, e.g. Anti-Biotin-FITC (# 130-090-756), and continue as described in step 4 to 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-PTK7 (CCK-4) antibodies

Aliquots of human PBMCs were stained with (a) Anti-PTK7 (CCK-4)-PE or (b) Anti-PTK7 (CCK-4)-Biotin/Anti-Biotin-PE, and CD141 (BDCA-3)-APC (# 130-090-907), CD303 (BDCA-2)-FITC (# 130-091-510) or CD34-FITC (# 130-081-001), respectively. Cells were analyzed by flow cytometry. Plasmacytoid dendritic cells (PDCs) are identified by CD303 (BDCA-2), type-2 myeloid dendritic cells (MDC2s) by CD141 (BDCA-3), and hematopoietic progenitor cells (HPCs) by CD34 expression. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. A. Fuchs , Colonna M.; manuscript in preparation

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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