

Anti-PTK7 (CCK-4) antibodies human

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

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1. Description

Components	1 mL Anti-PTK7 (CCK-4) antibodies, human: monoclonal anti-PTK7 (CCK-4) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	188B (isotype: mouse IgG2a).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

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.^{1,2,3} PTK7 (CCK-4) was identified to be important for neural development as a regulator of planar cell polarity, being required for convergent extension movement and neural tube closure.⁴ The PTK7 (CCK-4) gene is located on chromosome 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.⁵ 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).

1.2 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).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-PTK7 (CCK-4) conjugate	PE	APC	Biotin	pure
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11
- Anti-PTK7 (CCK-4) MicroBead-labeled cells	1:11	1:11	1:11	1:11
Immunohistochemistry^c				

a) The indicated antibody dilutions are for up to 1×10⁸ cells/mL of buffer.
 b) For optimal results, cells must be stained prior to fixation.
 c) The optimal antibody dilution should be determined by the user.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °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 bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.
- (Optional) Mouse IgG2a-FITC (# 130-091-837), Mouse IgG2a-PE (# 130-091-835), or Mouse IgG2a-APC (# 130-091-836) for isotype control.

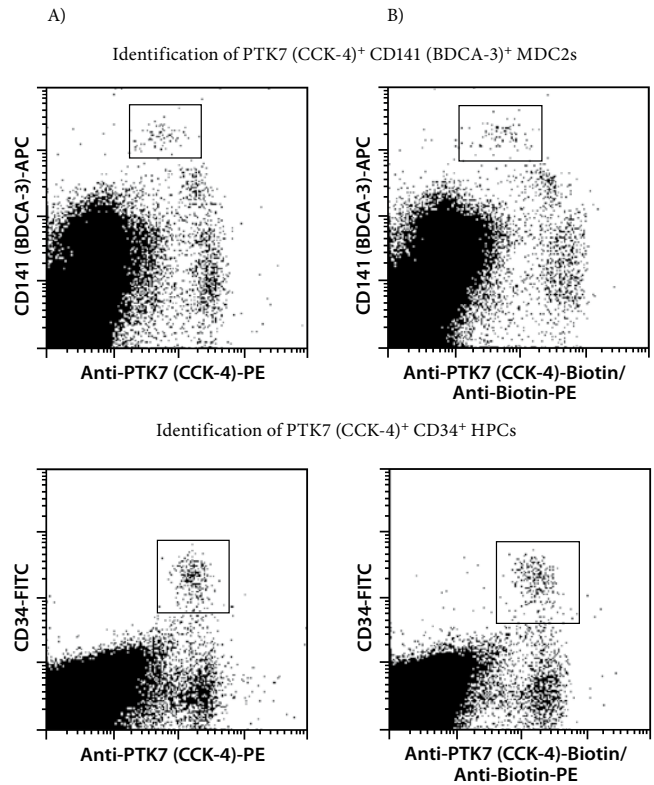
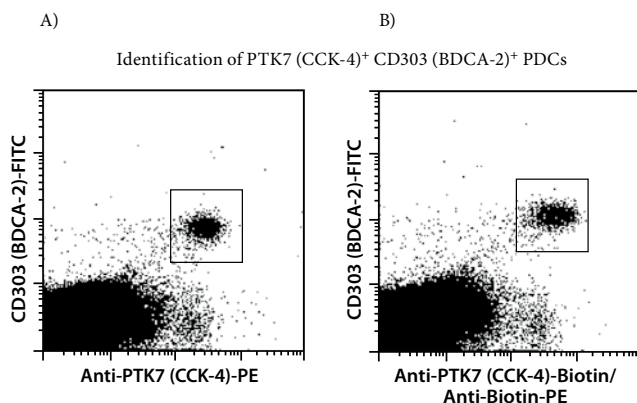
2. General protocol for immunofluorescent staining

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

1. Resuspend up to 10^7 cells in 80 μ L of buffer.
2. Add 20 μ L of FcR Blocking Reagent.
3. Add 10 μ L of the Anti-PTK7 (CCK-4) antibody.
4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
6. (Optional) If Anti-PTK7 (CCK-4)-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 4 and 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 peripheral blood mononuclear cells (PBMCs) were stained with Anti-PTK7 (CCK-4)-PE (A) or Anti-PTK7 (CCK-4)-Biotin/Anti-Biotin-PE (B) as well as CD141 (BDCA-3)-APC (# 130-090-907), CD303 (BDCA-2)-FITC (# 130-091-510), or CD34-FITC (# 130-081-001), and 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 propidium iodide fluorescence.



4. References

1. Mossie, K. *et al.* (1995) Colon carcinoma kinase-4 defines a new subclass of the receptor tyrosine kinase family. *Oncogene* 11: 2179–2184.
2. Park, S.-K. *et al.* (1996) Characterization of the Human Full-Length PTK7 cDNA Encoding a Receptor Protein Tyrosine Kinase-Like Molecule Closely Related to Chick KLG. *J. Biochem.* 119: 235–239.
3. Katoh, M. and Katoh, M. (2007) Comparative integromics on non-canonical WNT or planar cell polarity signaling molecules: transcriptional mechanism of PTK7 in colorectal cancer and that of SEMA6A in undifferentiated ES cells. *Int. J. Mol. Med.* 20: 405–409.
4. Lu, X. *et al.* (2004) PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature* 430: 93–98.
5. Xiao, Z. *et al.* (2008) Cell-specific internalization study of an aptamer from whole cell selection. *Chemistry* 14: 1769–1775.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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