Miltenyi Biotec

Anti-PTK7 (CCK-4) MicroBead Kit
human

Order no. 130-091-367

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1. Description

Components	**2 mL FcR Blocking Reagent:** Human IgG. **2 mL Anti-PTK7 (CCK-4) MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human PTK7 (CCK-4) antibodies (isotype: mouse IgG2a).
Size	For 2×10^9 total cells, up to 20 separations.
Product format	FcR Blocking Reagent is supplied as a solution containing stabilizer and 0.05% sodium azide. MACS MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First the PTK7 (CCK-4)$^+$ cells are magnetically labeled with Anti-PTK7 (CCK-4) MicroBeads. Then the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled PTK7 (CCK-4)$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of PTK7 (CCK-4)$^+$ cells. After removal of the column from the magnetic field, the magnetically retained PTK7 (CCK-4)$^+$ cells can be eluted as the positively selected cell fraction. The eluted PTK7 (CCK-4)$^+$ cells are separated once more over a new column to achieve highest purities.

1.2 Background and product applications

The Anti-PTK7 (CCK-4) MicroBead Kit is developed for the isolation of PTK7 (CCK-4)$^+$ cells from human peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMMNCs). 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 CD303 (BDCA-2)$^+$ plasmacytoid dendritic cells (PDCs),[2,3] CD141 (BDCA-3)^{high} type-2 myeloid dendritic cells (MDC2s),[2] and CD34$^+$ hematopoietic progenitor cells (HPCs).[1] PTK7 (CCK-4) was detected on early (CD34$^+$ CD133$^+$) and late (CD34$^+$ CD133$^-$) HPCs. 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).

Examples of applications

- Positive selection or depletion of PTK7 (CCK-4)$^+$ cells from blood, body fluids (e.g. bronchial lavage) or single-cell suspensions of bone marrow or tissue (e.g. lymphoid and tumor tissue).
- Simultaneous isolation or depletion of PDCs, MDC2s and HPCs from PBMCs or BMMNCs.
- Isolation or depletion of PDCs in case the CD304 (BDCA-4/Neuropilin-1) MicroBead Kit, human (# 130-090-532), or CD303 (BDCA-2) MicroBead Kit, human (# 130-090-509), cannot be used.
- Isolation or depletion of HPCs in case the CD34 MicroBead Kit, human (# 130-046-702) cannot be used.
- In combination with the CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit (#130-090-506) for isolation or depletion of dendritic cells and HPCs from PBMCs or BMMNCs.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) 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 (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.

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- MACS Columns and MACS Separators: PTK7 (CCK-4)$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection), or depleted by using LD, CS or D Columns. Positive selection or depletion can also be performed by using the autoMACS Separator

Column	max. number of labeled cells	max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS™ Separator

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric directed against:
  PTK7 (CCK-4), e.g. Anti-PTK7 (CCK-4)-PE, # 130-091-364; or -APC, # 130-091-366;
  CD303 (BDCA-2), e.g. CD303 (BDCA-2)-FITC, #130-090-510, or -PE, # 130-090-511, or -APC, # 130-090-905;
  CD141 (BDCA-3), e.g. CD141 (BDCA-3)-FITC, # 130-090-513, or -PE, # 130-090-514, or -APC, # 130-090-907;
  CD34, e.g. CD34-FITC, # 130-081-001, or -PE, # 130-081-002, or -APC, # 130-090-954.
- (Optional) PI (propidium iodide) or 7-AAD for flow- cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation (e.g. Ficoll-Paque™, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with bone marrow or tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. Remove dead cells by density gradient centrifugation or by using the Dead Cell Removal Kit (# 130-090-101).

2.2 Magnetic labeling

▲ Work fast, keep the cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 total cells. When working with fewer than 10^8 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 2×10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of **FcR Blocking Reagent** per 10^8 total cells.
5. Add 100 µL of **Anti-PTK7 (CCK-4) MicroBeads** per 10^8 total cells.
6. Mix well and incubate for 15 minutes at 4–8 °C.
   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
7. (Optional) Add staining antibodies, e.g. CD303 (BDCA-2)-PE (# 130-090-511), CD141 (BDCA-3)-APC (# 130-090-907) and CD34-FITC (# 130-081-001), in a dilution of 1:11, and incubate for additional 5 minutes at 4–8 °C.
8. Wash cells by adding 10–20 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
9. Resuspend up to 10^8 total cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 total cells in 500 µL of buffer.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of PTK7 (CCK-4)$^+$ cells (see table 1.3).

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL.
3. Apply cell suspension onto the column.

4. Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL.
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL.
7. Repeat the magnetic separation procedure as described in step 1– 6 using a new freshly prepared MACS Column.

   ▲ **Note:** If an MS Column was used for the first column run, the labeled cells may be directly eluted onto the second, equilibrated column. If an LS Column was used for the first column run, centrifuge cells after elution for 10 minutes (300×g), resuspend the cell pellet in 500 µL of buffer, and apply cell suspension onto the second, equilibrated column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "LD Column data sheet").
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (see "CS Column data sheet").
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way-stopcock of the assembled column (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 30 mL buffer from the top. Collect total effluent. This is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation, refer to the "D Column data sheet".

Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Posseld"

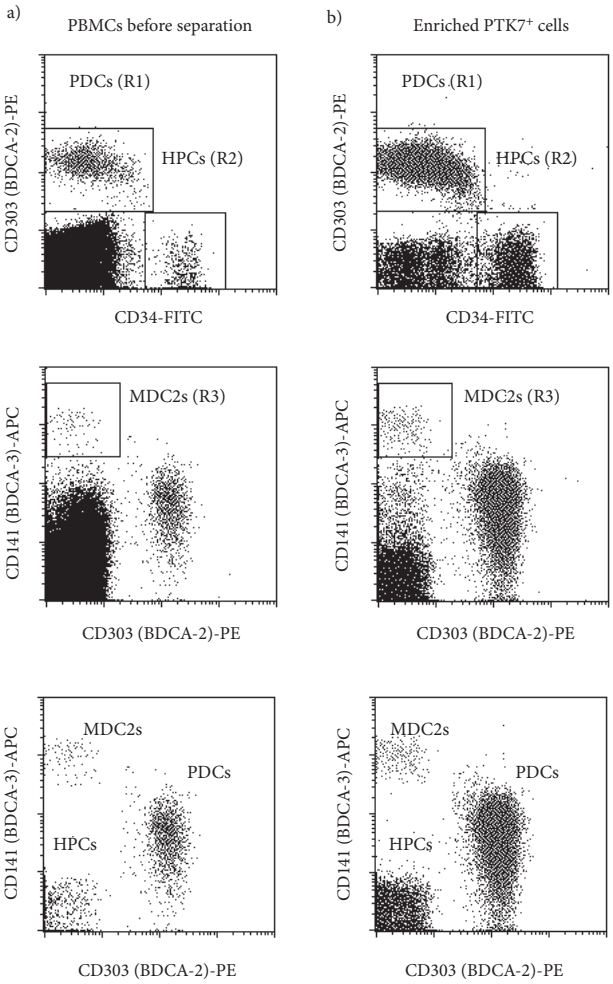
   Depletion: "Depletes"

   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Posseld", collect positive fraction (outlet port "pos2"). This is the purified PTK7 (CCK-4)$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the PTK7 (CCK-4)$^-$ cell fraction.

3. Example of a separation using Anti-PTK7 (CCK-4) MicroBead Kit

Separation of PBMCs using the Anti-PTK7 (CCK-4) MicroBead Kit and a MiniMACS™ Separator with two MS Columns. Aliquots are fluorescently stained (a) before separation and (b) after magnetic separation with CD34-FITC, CD303 (BDCA-2)-PE and CD141 (BDCA-3)-APC for identification of hematopoietic progenitor cells (HPCs), plasmacytoid dendritic cells (PDCs) and type-2 myeloid dendritic cells (MDC2s), respectively. To control that there is no overlap between the different PTK7 (CCK-4)-expressing cell types, cells were finally gated on R1 (PDCs), R2 (HPCs) and R3 (MDC2s). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Fuchs A. and Colonna M.; manuscript in preparation.
2. Dzionek A. *et al.* (2000) BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. 165: 6037-6046. [898]
3. Dzionek A. *et al.* (2002) Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions. Hum. Immunol. 63: 1133–1148. [2423]

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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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