Miltenyi Biotec

CD184 (CXCR4) MicroBead Kit
human

Order no. 130-100-070

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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.

1. Description

This product is for research use only.

Components	**200 µL CD184 (CXCR4)-APC, human:** monoclonal CD184 (CXCR4) antibody conjugated to APC (isotype: mouse IgG2a). **2 mL Anti-APC MicroBeads:** MicroBeads conjugated to monoclonal mouse anti-APC antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All reagents 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 Principle of the MACS® Separation

First, the CD184 (CXCR4)$^+$ cells are magnetically labeled with CD184 (CXCR4)-APC and Anti-APC 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 CD184 (CXCR4)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD184 (CXCR4)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD184 (CXCR4)$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD184 is also known as CXCR4 or fusin, a 45 kDa seven transmembrane G-protein-linked chemokine receptor for SDF-1. CD184 is expressed on Sox17$^+$/FoxA2$^+$ definitive endoderm (DE) cells that can be derived from pluripotent stem cells (PSCs) *in vitro*. DE differentiation is induced by high doses of activin A in combination with Wnt and FGF pathway activation. 3-5 days after induction DE cells can be isolated based on CD184.

1.3 Applications

- Positive selection of cells expressing human CD184 (CXCR4) antigen.
- Isolation of CD184 (CXCR4)$^+$ early endoderm cells derived from human pluorpotent stem cells.

1.4 Reagent and instrument requirements

- PB buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+} and 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- PEB 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). Degas buffer before use, as air bubbles could block the column.

  ▲ **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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. Positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^7	2×10^7	MidiMACS, QuadroMACS, SuperMACS II
autoMACS	2×10^7	4×10^7	autoMACS Pro

  ▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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2. Protocol

2.1 Sample preparation

For this protocol following reagents are needed: DPBS buffer (Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}), soybean trypsin inhibitor, DNase (225 U/mL).

1. Remove culture medium and wash culture plates twice with DPBS buffer.
2. Harvest cells by adding 2 mL of trypsin/EDTA solution (0.05% trypsin, 2 mM EDTA) per 10 cm culture dish for 3 minutes at 37 °C.
3. Stop enzymatic reaction by addition of soybean trypsin inhibitor supplemented with 225 U/mL DNase.
4. Transfer cell suspension into an appropriate vial.
5. Wash culture dish once with 5 mL PB-buffer/DNase and add to the previously collected cell suspension.



2.2 Magnetic labeling

▲ Work fast, keep 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 10^7 total 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 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 labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of appropriate differentiation medium or PEB buffer per 10^7 total cells.
4. Add 2 µL of CD184 (CXCR4)-APC per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of appropriate differentiation medium or PEB buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of appropriate differentiation medium or PEB buffer per 10^7 total cells.
8. Add 20 µL of Anti-APC MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of appropriate differentiation medium or PEB buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
11. Resuspend up to 2×10^7 cells in 500 µL of appropriate differentiation medium or PB buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. 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 CD184 $(CXCR4)^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare LS Column by rinsing with 3 mL of PEB buffer or culture medium.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of PEB buffer or culture medium. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of PEB buffer or appropriate culture medium onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

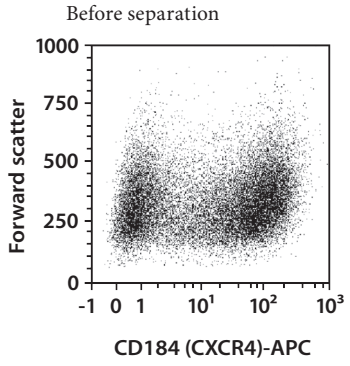
   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

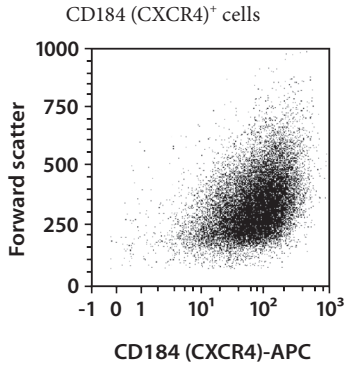
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3. Example of a separation using the CD184 (CXCR4) MicroBead Kit

CD184 (CXCR4)$^+$ cells were isolated from human pluripotent stem cell-derived definitive endoderm cells using the CD184 (CXCR4) MicroBead Kit human, an LS Column, and a MidiMACS™ Separator. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





4. References

1. Wang, P. *et al.* (2011) Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm. Cell Stem Cell. 8(3): 335–346.
2. Bone, H. K. *et al.* (2011) A novel chemically directed route for the generation of definitive endoderm from human embryonic stem cells based on inhibition of GSK-3. J. Cell Sci. 124(Pt 12): 1992–2000.
3. Cheng, X. *et al.* (2012) Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. Cell Stem Cell. 10(4): 371–384.
4. D'Amour, K. A. *et al.* (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat. Biotechnol. 23(12): 1534–1541.

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.

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