Miltenyi Biotec

CD309 (Flk-1) MicroBead Kit
mouse

Order no. 130-097-346

Contents

1. Description

Components
2 mL CD309-Biotin, mouse:
monoclonal CD309 antibody conjugated to Biotin (isotype: rat IgG2a).
2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: rat 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 CD309$^+$ cells are indirectly magnetically labeled with CD309-Biotin antibodies and Anti-Biotin 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 CD309$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD309$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD309$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD309 is a class 3 receptor tyrosine kinase, also known as fetal liver kinase 1 (Flk-1), vascular endothelial growth factor receptor 2 (VEGFR2), or kinase insert domain receptor (KDR). CD309 is one of the earliest differentiation markers for endothelial and hematopoietic cells.[1,2] Utilizing murine embryonic stem cells it has been shown that CD309 is a marker for multipotent cardiovascular progenitor cells that give rise to cardiomyocyte, endothelial, and vascular smooth muscle lineages.[3,4]

1.3 Applications

- Positive selection of cells expressing the mouse CD309 antigen.
- Positive selection of CD309$^+$ multipotent cardiovascular progenitor cells derived from pluripotent stem cells.
- Positive selection of hematopoietic precursor cells expressing the mouse CD309 antigen.

1.4 Reagent and instrument 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).
- MACS Columns and MACS Separators: CD309$^+$ cells can be enriched by using LS Columns. Positive selection can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated Labeling Check Reagents for flow cytometric analysis, e.g., Labeling Check Reagent-APC (# 130-095-237). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.
- (Optional) Embryoid Body Dissociation Kit, human and mouse (# 130-096-348)

2. Protocol

2.1 Sample preparation

When differentiating via embroid bodies, the Embryoid Body Dissociation Kit, human and mouse (# 130-096-348) is recommended for generation of single-cell suspensions.

▲ **Note:** When using the Embryoid Body Dissociation Kit, prepare enzyme mix 1 as described in the note of section 2.1, step 2.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

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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, # 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 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD309-Biotin per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. (Optional) Resuspend cell pellet in 100 µL buffer, add 10 µL staining antibodies, e.g., 10 µL of Labeling Check Reagent-APC (# 130-095-237), mix well, incubate for 10 minutes in the dark in the refrigerator (2–8 °C), wash cells by adding 1-2 mL of buffer, and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

    ▲ **Note:** Labeling Check Reagent guarantees optimal flow cytometric analysis of isolated $CD309^+$ cells. Use of Anti-Biotin conjugates or CD309 antibodies is not recommended.
12. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
13. 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 $CD309^+$ 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 column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

    ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer 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 or the autoMACS® Separator

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

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

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 the following program:

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

Magnetic separation with the autoMACS® Separator

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 at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

    Positive selection: Possels
    Collect positive fraction from outlet port pos1.

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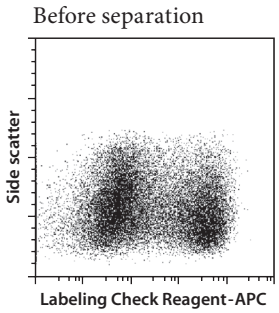
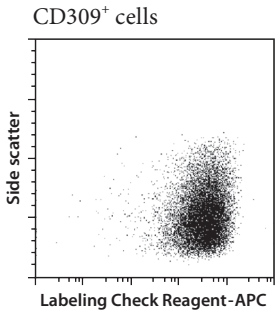
3. Example of a separation using the CD309 (Flk-1) MicroBead Kit

Murine embryonic stem cells (HM1 line) were differentiated via embryoid bodies for 5 days. Embryoid bodies were dissociated using the Embryoid Body Dissociation Kit (# 130-096-348) and CD309 expressing cells were isolated using the CD309 (Flk-1) MicroBead Kit, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with Labeling Check Reagent-APC (# 130-095-237) and 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. Shalaby, F. *et al.* (1995) Failure of blood-island formation and vasculogenesis in Flk1-deficient mice. Nature 376: 62–66.
2. Eichmann, A. *et al.* (1997) Ligand-dependent of the endothelial and hematopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor 2. Proc. Natl. Acad. Sci. 94: 5141–5146.
3. Kattman, S.J. *et al.* (2006) Multipotent flk-1$^+$ cardiovascular progenitor cells give rise to cardiomyocyte, endothelial, and vascular smooth muscle lineages. Dev. Cell 5: 723–732.
4. Joo, H.J. *et al.* (2012) ROCK suppression promotes differentiation and expansion of endothelial cells from embryonic stem cell–derived Flk1$^+$ mesodermal precursor cells. Blood 120: 2733–2744.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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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