



Miltenyi Biotec

# Myeloid-Derived Suppressor Cell Isolation Kit

## mouse

Order no. 130-094-538



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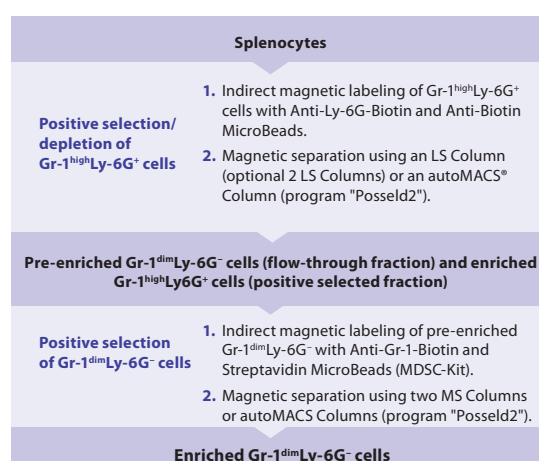
### 1. Description

<b>Components</b>	<b>2 mL Anti-Ly-6G-Biotin (MDSC-Kit), mouse:</b> monoclonal antibodies conjugated to biotin (isotype: rat IgG2a). <b>2 mL Anti-Gr-1-Biotin (MDSC-Kit), mouse:</b> monoclonal antibodies conjugated to biotin (isotype: rat IgG2b). <b>2×2 mL Anti-Biotin MicroBeads:</b> MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). <b>2 mL Streptavidin MicroBeads (MDSC-Kit):</b> MicroBeads conjugated to streptavidin. <b>1 mL FcR Blocking Reagent, mouse</b>
<b>Capacity</b>	For 2×10 <sup>9</sup> total cells, up to 20 separations.
<b>Product format</b>	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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

The Myeloid-Derived Suppressor Cell Isolation Kit has been developed for the isolation of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> and/or Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells. First, Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells are indirectly magnetically labeled with Anti-Ly-6G-Biotin (MDSC-Kit) 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 Ly-6G<sup>+</sup> cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> and pre-enriched for Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells. After removing the column from the magnetic field, the magnetically retained Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the Gr-1<sup>high</sup>Ly-6G<sup>+</sup> myeloid cells is separated over a second column. Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells that are contained in the first flow through fraction are indirectly magnetically labeled with Anti-Gr-1-Biotin (MDSC-Kit) and Streptavidin MicroBeads (MDSC-Kit) and isolated by positive selection from the pre-enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cell fraction. The magnetically labeled Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells are retained on the column and eluted after removal of the column from the magnetic field. To increase the purity, the positively selected cell fraction containing the Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells is separated over a second column.



## 1.2 Background information

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells with a remarkable capacity to induce T cell dysfunction. Phenotypically characterized as CD11b<sup>+</sup>Gr-1<sup>+</sup>-expressing cells, partial blocking of the cell differentiation of immature myeloid progenitor cells into granulocytes, macrophages, or dendritic cells results in the expansion of this population in various pathological conditions (cancer, infection, autoimmune diseases). Activation of MDSCs causes an increased production of immune suppressive factors Arginase 1 and iNOS, reactive oxygen species (ROS) and nitric oxide (NO) and thereby influences the T cell proliferation capacity, induces T cell apoptosis, or lead to unresponsiveness of T cells.<sup>1-5</sup>

The ability to suppress T cell function by MDSCs isolated with the Myeloid-Derived Suppressor Cell Isolation Kit was determined using CT26, 4T1, MCA203, and EL-4 tumor models in BALB/c and C57BL/6 mice, respectively.

The Myeloid-Derived Suppressor Cell Isolation Kit has been developed for the isolation of polymorphonuclear Gr-1<sup>high</sup>Ly-6G<sup>+</sup> and mononuclear Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> myeloid cells.<sup>6</sup> Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells can be further subdivided according to the expression of the marker Ly-6C into Gr-1<sup>dim</sup>Ly-6C<sup>high</sup>Ly-6G<sup>-</sup> and Gr-1<sup>dim</sup>Ly-6C<sup>low</sup>Ly-6G<sup>-</sup> cells. The proportion between Ly-6C<sup>high</sup> and Ly-6C<sup>low</sup> subpopulations depends on the used tumor models.

## 1.3 Applications

- Isolation of MDSCs from spleen and tumors.
  - ▲ **Note:** The Myeloid-Derived Suppressor Cell Isolation Kit is not suitable for the isolation of MDSCs from bone marrow.
- Isolation of MDSCs for the analysis of their functional properties, e.g., on T cell activation and effector functions in different experimental mouse models.
- Isolation of MDSCs for phenotypical analysis.
- Isolation of MDSCs for downstream analysis such as gene expression profiling.

## 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). 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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- MACS® Columns and MACS Separators: Positive selection of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells can be performed by using two LS Columns. Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells can be enriched by first depletion of Ly-6G<sup>+</sup> cells by using an LS Column and subsequent positive selection of Gr-1<sup>+</sup> cells by using two MS Columns. Positive selection or depletion 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
<b>Positive selection</b>			
LS	10 <sup>8</sup>	2×10 <sup>9</sup>	MidiMACS, QuadroMACS, SuperMACS
<b>Second positive selection</b>			
MS	10 <sup>7</sup>	2×10 <sup>8</sup>	MiniMACS, OctoMACS, SuperMACS
<b>Positive selection</b>			
autoMACS	2×10 <sup>8</sup>	4×10 <sup>9</sup>	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-Ly-6C-FITC, Anti-Ly-6G-PE, Anti-Gr-1-PE, and CD11b-APC. For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Propidium Iodide Solution (# 130-093-233) or

7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

## 2. Protocol

### 2.1 Sample preparation

Prepare a single-cell suspension from spleen and tumors using a standard preparation method. For details see the protocols section at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

▲ The Myeloid-Derived Suppressor Cell Isolation Kit, mouse is not suitable for isolation of MDSCs from bone marrow.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

### 2.2 Magnetic labeling of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells

▲ 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<sup>8</sup> total cells. When working with fewer than 10<sup>8</sup> 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<sup>8</sup> 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. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

- Determine cell number.
- Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
- Resuspend cell pellet in 350 µL of buffer per 10<sup>8</sup> total cells.
- Add 50 µL of FcR Blocking Reagent per 10<sup>8</sup> total cells.
- Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
- Add 100 µL of Anti-Ly-6G-Biotin (MDSC-Kit).
- Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
- Wash cells by adding 5–10 mL of buffer per 10<sup>8</sup> cells and centrifuge

at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.

- Resuspend up to 10<sup>8</sup> cells in 800 µL of buffer.
  - Add 200 µL of Anti-Biotin MicroBeads.
  - Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
  - Wash cells by adding 10–20 mL of buffer per 10<sup>8</sup> cells and centrifuge at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
  - Resuspend up to 10<sup>8</sup> cells in 500 µL of buffer.
- ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
- Proceed to magnetic separation (2.3).

### 2.3 Magnetic separation: Positive selection of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells

▲ Choose an appropriate MACS® Column and MACS Separator according to the number of total cells and the number of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells. For details see table in section 1.4.

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

#### Magnetic separation with LS Columns

- Place LS Column in the magnetic field of a suitable MACS Separator. For details see 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 effluent from step 3; this is the unlabeled pre-enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cell fraction.  
▲ **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.
7. To increase the purity of Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells, the eluted fraction should be enriched over a second LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.
8. Proceed to 2.4 for further isolation of Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells.

#### Magnetic separation with the autoMACS<sup>®</sup> 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  $\geq 10^{\circ}\text{C}$ .
- ▲ 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 program: "Posseld2"  
Collect negative fraction in row B of the tube rack. This fraction represents the unlabeled and pre-enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells.  
Collect positive fraction in row C of the tube rack.  
This fraction represents the magnetically labeled Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells.

#### Magnetic separation with the autoMACS<sup>®</sup> 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 pos2.
3. For a standard separation choose the program: "Posseld2"  
Collect negative fraction from outlet port neg1. This fraction represents the unlabeled and pre-enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells.  
Collect positive fraction outlet port pos2.  
This fraction represents the magnetically labeled Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells.

#### 2.4 Magnetic labeling of Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of 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  $2 \times 10^8$  total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Centrifuge cell suspension at  $300 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ . Aspirate supernatant completely.
2. Resuspend cell pellet in 400  $\mu\text{L}$  of buffer per  $10^8$  total cells.

3. Add 100  $\mu\text{L}$  of Anti-Gr-1-Biotin (MDSC-Kit) per  $10^8$  total cells.
4. Mix well and incubate for 10 minutes in the refrigerator ( $2-8^{\circ}\text{C}$ ).
5. Wash cells by adding 5–10 mL of buffer per  $10^8$  cells and centrifuge at  $300 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ . Aspirate supernatant completely.
6. Resuspend cell pellet in 900  $\mu\text{L}$  of buffer per  $10^8$  total cells.
7. Add 100  $\mu\text{L}$  of Streptavidin MicroBeads (MDSC-Kit).
8. Mix well and incubate for 15 minutes in the refrigerator ( $2-8^{\circ}\text{C}$ ).
9. Wash cells by adding 10–20 mL of buffer per  $10^8$  cells and centrifuge at  $300 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ . Aspirate supernatant completely.
10. Resuspend up to  $10^8$  cells in 500  $\mu\text{L}$  of buffer.  
▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.5).

#### 2.5 Magnetic separation: Positive selection of Gr-1<sup>dim</sup>Ly6G<sup>-</sup> cells

▲ To achieve highest purities, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS<sup>®</sup> Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500  $\mu\text{L}$  of buffer.
3. Apply cell suspension onto the column.

4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction.

▲ **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.

▲ **Note:** To perform a second column run, cells may be eluted directly from the first onto the second, equilibrated column instead of a collection tube.

6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells (Gr-1<sup>dim</sup>Ly-6G<sup>-</sup>) by firmly pushing the plunger into the column.
7. To increase purity of Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells, enrich the eluted fraction over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

#### Magnetic separation with the autoMACS<sup>®</sup> 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 program: "Posseld2"  
Collect positive fraction in row C of the tube rack. This fraction represents the magnetically labeled Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells.

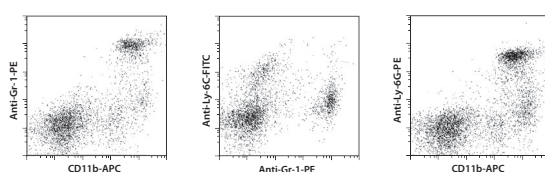
#### Magnetic separation with the autoMACS Separator

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 pos2.
3. For a standard separation choose the program: "Posseld2"  
Collect positive fraction from outlet port pos2. This fraction represents the magnetically labeled Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells.

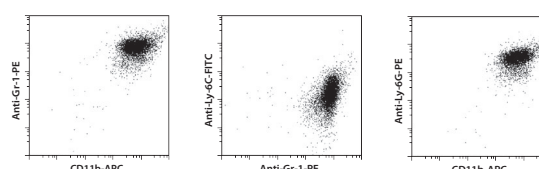
### 3. Example of a separation using the Myeloid-Derived Suppressor Cell Isolation Kit

Myeloid-derived suppressor cells were isolated from a spleen of a BALB/c mouse using the Myeloid-Derived Suppressor Cell Isolation Kit, two LS Columns, two MS Columns, and an appropriate MACS<sup>®</sup> Separator. Cells were fluorescently stained with Anti-Ly-6C-FITC, CD11b-APC, and Anti-Gr-1-PE or Anti-Ly-6G-PE, respectively, and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

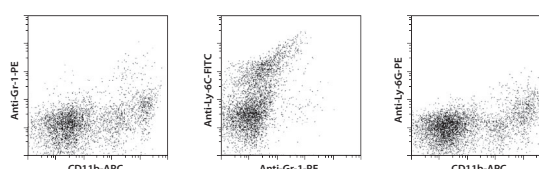
Spleen cells before separation



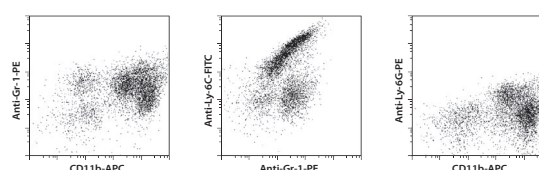
Enriched Gr-1<sup>high</sup>Ly-6G<sup>+</sup> cells



Pre-enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells



Enriched Gr-1<sup>dim</sup>Ly-6G<sup>-</sup> cells



## 4. References

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