Miltenyi Biotec

StraightFrom® Spleen CD8a MicroBead Kit
mouse

Order no. 130-130-149

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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	**2 mL StraightFrom Spleen CD8a MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-mouse CD8a antibodies (isotype: rat IgG2a). **13 mL Buffer S (20× Stock Solution)** **1 vial of Enzyme A (lyophilized powder)**
Capacity	For 40 spleens. The specified number of digestions is valid when digesting a spleen with an average weight of 80–140 mg following the protocol in chapter 2.2.
Product format	StraightFrom Spleen CD8a MicroBeads, mouse are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Upon arrival store all components at 2–8 °C. Reconstitute Enzyme A before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1. The expiration date is indicated on the vial label.

1.1 Principle of the StraightFrom Spleen Separation

Spleen is enzymatically digested using the kit components, and the gentleMACS™ Dissociators are used for the mechanical dissociation steps. During the dissociation step $CD8a^+$ cells are magnetically labeled with StraightFrom Spleen CD8a MicroBeads, mouse. After simultaneous dissociation and magnetic labeling, the sample is passed through a 70 µm filter to remove larger particles from the single-cell suspension. Then, the cell suspension is loaded onto MACS® Column placed in the magnetic field of a MACS Separator. The magnetically labeled $CD8a^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD8a^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD8a^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The StraightFrom Spleen CD8a MicroBead Kit, mouse has been developed for the positive selection of $CD8a^+$ cells directly from mouse spleen by using the gentleMACS Dissociators in combination with enzymatic digestion and MACS Technology. Spleen is gently dissociated into single-cell suspension and $CD8a^+$ cells are magnetically labeled simultaneously. No cell count is required as well as no washing step after labeling, leading to purification of viable and functionally active cells that can directly be used for downstream experiments. In mouse spleen, the CD8a antigen is expressed on almost all cytotoxic T cells and on subpopulations of dendritic cells. CD8a functions as an accessory molecule in the recognition of MHC class I/peptide complexes by the TCR heterodimer on cytotoxic $CD8a^+$ T cells.

1.4 Applications

- Positive selection of $CD8a^+$ T cells from spleen.
- Isolation of purified $CD8a^+$ cells for *in vitro* and *in vivo* studies, e.g., on protective immune responses against parasites or allergens, and for adoptive transfer into immunodeficient and virus infected mice.

1.5 Reagent and instrument requirements

- Separation 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). Always use freshly prepared buffer. Do not use autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results. Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** 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.

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- Pre-Separation Filters (70 µm) (# 130-095-823)
- gentleMACS Octo Dissociator (# 130-095-937) or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- MACS Columns and MACS Separators: $CD8a^+$ cells can be enriched by using LS Columns. Positive selection can also be performed by using the MultiMACS™ Cell24 Separator Plus or autoMACS Columns on the autoMACS NEO or autoMACS Pro Separators.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
	10^8	10^9	MultiMACS Cell24 Separator Plus
MS*	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS NEO Separator, autoMACS Pro Separator
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

* Use MS Columns only after first magnetic separation with LS Columns.

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

▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4 Antibody, anti-mouse, VioBlue®, REAfinity™, and CD8a Antibody, anti-mouse, PE, REAfinity. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

2. Protocol

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ One mouse spleen is dissociated in a volume of approximately 1 mL enzyme-MicroBead–mix. The weight of one mouse spleen amounts to 80–140 mg (female BALB/c mouse, 6–7 weeks old).

▲ Remove fat tissue from the dissected mouse spleen before dissociation.

2.1 Reagent preparation

1. Prepare 1× Buffer S by adding, for example, 0.25 mL of 20× Buffer S to 4.75 mL of sterile, double-distilled water for one spleen. Store at 2–8 °C.

   ▲ **Note:** Handle under sterile conditions.

2. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of 1× Buffer S. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

2.2 Spleen dissociation and magnetic labeling of 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 given below are for one spleen. When working with more spleens (max. of three spleens) per C Tube, scale up all reagent volumes accordingly (e.g. for two spleens, use twice the volume of all indicated reagent volumes).

1. Prepare the enzyme-MicroBead–mix by adding 930 µL 1× Buffer S, 20 µL Enzyme A, and 50 µL StraightFrom Spleen CD8a MicroBeads, mouse into a gentleMACS C Tube.
2. Transfer one mouse spleen into the gentleMACS C Tube containing the enzyme-MicroBead–mix.

   ▲ **Note:** If the spleen has been stored in buffer with chelating agent, e.g., EDTA, rinse spleen thoroughly, e.g., with 1× Buffer S or PBS. Remove excess buffer.
3. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.

   ▲ **Note:** Ensure that the sample material is located in the area of the rotor/stator.
4. Run the gentleMACS Program **m_SF_SMBK_01**.
5. After termination of the program, detach the C Tube from the gentleMACS Dissociator.

   ▲ **Note:** The spleen will not be completely dissociated after this step. In the unexpected event that the spleen is not dissociated at all, repeat steps 4 and 5.
6. Perform a short centrifugation step to collect the sample material at the bottom of the tube.

   ▲ **Note:** Do not exceed 300×g speed.

   ▲ **Note:** If a cell pellet has formed, carefully resuspend the cells by pitteting up and down.
7. Place a Pre-Separation Filter (70 µm) on a 15 mL tube. Moisturize the Pre-Separation Filter (70 µm) by pipetting 0.5 mL of 1× Buffer S into the reservoir and discard effluent.
8. Apply cell suspension on top of the Pre-Separation Filter.
9. Apply 0.5 mL of separation buffer on the Pre-Separation Filter. Tap the rim of the filter to elute the suspension completely.
10. Proceed directly 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 $CD8a^+$ 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.

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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 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 buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of CD8a$^+$ cells, the eluted fraction can be enriched over a second LS Column or a MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with the MultiMACS Cell24 Separator

Refer to the the MultiMACS Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

2.4 Magnetic separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

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

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction, position C = labeled (positive) fraction.

2.4.1 Magnetic separation using the autoMACS NEO Separator

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Magnetic separation after manual labeling

1. Dissociate and label the sample as described in section 2.2 Spleen dissociation and magnetic labeling of cells.
2. Prepare and prime the instrument.
3. Place the Chill Rack on the MACS MiniSampler S.
4. Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
5. Assign a reagent to each sample.
6. Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
7. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
8. The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.
9. Place the sample(s) and empty tubes to the Chill Rack.
10. Tap **Run** to start the separation process.

2.4.2 Magnetic separation using the autoMACS Pro Separator

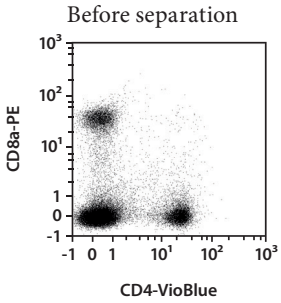
Magnetic separation after manual labeling

1. Dissociate and label the sample as described in section 2.2 Spleen dissociation and magnetic labeling of cells.
2. Prepare and prime the instrument.
3. Apply tube containing the sample.
4. For a standard separation choose the following program:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.
5. Tap **Run** to start the separation process.

3. Example of a separation using the StraightFrom Spleen CD8a MicroBead Kit, mouse

Separation of a spleen sample using the StraightFrom Spleen CD8a MicroBead Kit, mouse and the autoMACS NEO Separator. Cells were fluorescently stained with REAfinity Antibodies CD4-VioBlue and CD8a-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence.



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.

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