Anti-DC (OX62) MicroBeads
rat

Magnetic cell sorting

Order No. 130-090-663

Index

1. Description

Components	2 mL Anti-DC (OX62) MicroBeads, rat: MicroBeads conjugated to monoclonal mouse anti-DC (OX62) antibodies (isotype: mouse IgG1; clone: OX62).
Size	For 10^9 total cells, up to 100 separations.
Product format	The product is supplied as suspension containing 0.1% gelatine 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 OX62$^+$ cells are magnetically labeled with Anti-DC (OX62) 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 OX62$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of OX62$^+$ cells. After removal of the column from the magnetic field, the magnetically retained OX62$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Anti-DC (OX62) MicroBeads are developed for the positive selection or depletion of OX62$^+$ dendritic cells (DC) from blood, lymphoid and non-lymphoid tissue. The monoclonal antibody clone OX62 recognizes the integrin α_{E2} subunit[1] (molecular mass 150 kDa)[2] expressed on dendritic cells in the rat. Notably, OX62 antigen is found on both CD4$^+$ OX41$^+$ and CD4$^-$ OX41$^-$ dendritic cells.[3,4,5] The OX62 antigen was also shown to be expressed on MHC II$^-$ TCRγ/δ$^+$ T cells[1], and on a subset of folliculo-stellate like (S100$^+$) cells in the anterior pituitary[6,7]. OX62 is not detectable on Langerhans cells.[1]

Examples of applications

- Positive selection or depletion of dendritic cells from single-cell suspensions of lymphoid or non-lymphoid tissue, or peripheral blood.[8]

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA by diluting MACS BSA Stock Solution (#130-091-376) 1:20 in 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 rat serum albumin, rat serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: OX62$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). Anti-DC (OX62) MicroBeads can be used for depletion of OX62$^+$ cells on LD, CS or D Columns. Cells which strongly express the OX62 antigen can also be depleted using MS, LS or XS 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

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

- Collagenase D: 2 mg/mL Collagenase D (>0.15 U/ mg, e.g. from Roche, Germany) in RPMI/ 1% FCS.
- (Optional) Nycodenz®-PBS solution. Nycodenz® (from Nycomed As, Oslo, Norway) solubilized in PBS buffer at a final concentration of 14.5% (w/v).
- (Optional) Fluorochrome-conjugated Anti-DC (OX62) antibody for flow-cytometric analysis.

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- (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

Prepare a single-cell suspension from lymphoid organs, non-lymphoid tissue or peripheral blood using standard methods (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101) is recommended.

Preparation of rat spleens with Collagenase D

To increase the recovery and final purity of dendritic cells, it is recommended to prepare spleen cell suspensions by Collagenase D treatment:

1. Place up to 2 isolated spleens in a 6 cm petri-dish with sufficient Collagenase D to completely cover the bottom of the dish (about 5 mL).
2. Inject spleens with 500 µL of Collagenase D per spleen using a 1 mL syringe and a 25G needle and cut the tissue in smaller pieces using a pair of scissors and a pair of forceps.
3. Incubate spleen pieces for 25 minutes at 37 °C in Collagenase D. Add 10 mM EDTA and incubate a further 5 minutes.
4. Pass the digested material through a steel mesh using a plunger of a syringe and wash the petri-dish and the mesh with buffer.
5. Pass all cells through a Pre-Separation Filters (# 130-041-407) to remove clumps and collect cells in a 50 mL tube. Add buffer to a final volume of about 50 mL.
6. Wash cells by centrifugation at 200×g for 10 minutes. Count cells.
7. Proceed to Nycodenz gradient centrifugation or magnetic labeling (2.2)

(Optional) Pre-enrichment of rat dendritic cells by Nycodenz® gradient centrifugation

For highly pure dendritic cells, $OX62^+$ $CD3^+$ TCR γ/δ^+ T cells might be depleted prior to OX62 separation by Nycodenz® gradient centrifugation.

1. After centrifugation remove supernatant completely and resuspend cell pellet in 1 mL of buffer per $1–2\times10^8$ **total** cells. For fewer cells, use same volume.
2. Layer cell suspension on equal volume of 14.5% (w/v) Nycodenz-PBS solution (1.079 g/mL) and centrifuge for 13 minutes at 1,800×g and 4 °C.
3. Recover the low density cells at the top of the low density solution. Add buffer to a final volume of 50 mL and wash cells by centrifugation for 10 minutes at 200×g and 4 °C. Count cells.

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 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 cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Anti-Rat DC (OX62) MicroBeads per 10^7 total cells.
5. 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.
6. (Optional) Add staining antibodies and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. Resuspend up to 10^8 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 cells in 500 µL of buffer.
9. 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 $OX62^+$ cells (see table in section 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.

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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.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared 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: "Possel"

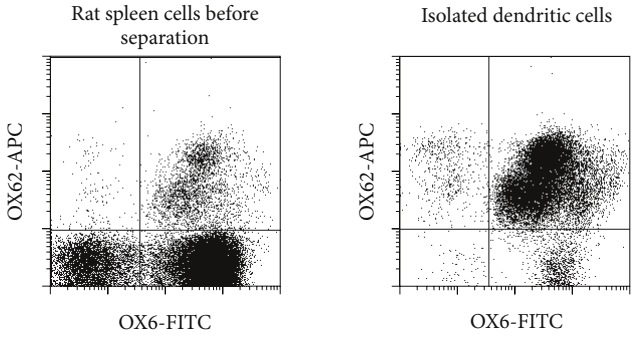
   Depletion: "Depletes"

   ▲ **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 "Possel", collect positive fraction (outlet port "pos1"). This is the purified OX62$^+$ cell fraction.

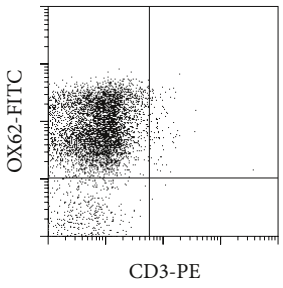
   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the OX62$^-$ cell fraction.

3. Example of a separation using Anti-DC (OX62) MicroBeads

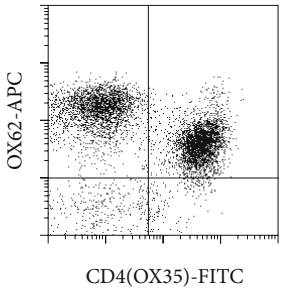
Dendritic cells were pre-enriched by Nycodenz gradient centrifugation from rat spleen cell suspension and were then isolated using Anti-DC (OX62) MicroBeads and a MiniMACS™ Separator with an MS Column. For flow cytometric analysis, cells were stained for OX62 and MHC Class II (OX6). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



To evaluate the depletion of OX62$^+$CD3$^+$TCR γ/δ T cells from rat spleen by Nycodenz-preparation followed by MACS Technology, a sample of the isolated dendritic cells was stained with CD3-PE.



To distinguish between the two major OX62$^+$ dendritic cells subsets a sample of the isolated dendritic cells was additionally stained for CD4 (OX35). Note that OX62^{bright} dendritic cells are CD4$^-$, whereas OX62^{dim} cells are CD4$^+$.



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5. References

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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.

Warranty

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