Miltenyi Biotec

CD140a (PDGFRα) MicroBead Kit
mouse

Order no. 130-101-502
Order no. 130-101-547

Contents

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	**50 µL CD140a (PDGFRα) MicroBeads, mouse (# 130-101-547)** or **1 mL CD140a (PDGFRα) MicroBeads, mouse (# 130-101-502):** MicroBeads conjugated to monoclonal CD140a (PDGFRα) antibodies (isotype: rat IgG2b). **50 µL FcR Blocking Reagent, mouse (# 130-101-547)** or **1 mL FcR Blocking Reagent, mouse (# 130-101-502)**
Capacity	For 5×10^7 total cells, up to 5 separations (# 130-101-547) or for 10^9 total cells, up to 100 separations (# 130-101-502).
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, Fc receptors are blocked with FcR Blocking Reagent, mouse. Then, the CD140a (PDGFRα)$^+$ cells are magnetically labeled with CD140a (PDGFRα) MicroBeads. The cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD140a (PDGFRα)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD140a (PDGFRα)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD140a (PDGFRα)$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected fraction containing the CD140a (PDGFRα)$^+$ cells can be separated over a second column.

1.2 Background information

CD140a (PDGFRα) MicroBeads (PDGFRα: platelet-derived growth factor receptor alpha) have been developed for the separation of mouse cells based on the expression of the CD140a (PDGFRα) antigen. CD140a (PDGFRα) has been reported to be broadly expressed in embryonic tissue, various malignancies and embryonic stem cell-derived cardiomyogenic cells.[1-2] Furthermore, CD140a (PDGFRα) is specifically expressed by oligodendrocyte precursor cells, which differentiate into myelinating oligodendrocytes.[3-5]
CD140a (PDGFRα) MicroBeads were especially optimized for the separation of oligodendrocyte progenitor cells (OPCs) based on the expression of CD140a (PDGFRα). The isolation was tested particularly on dissociated postnatal CD1 mouse brain tissue derived from animals younger than postnatal day eight (P8). In principle, cell isolation is also possible from older mice, but purity of the positive fraction might be lower due to lower frequency of CD140a (PDGFRα) positive cells after tissue dissociation.
For optimal results, the Neural Tissue Dissociation Kit (P) is recommended prior to cell isolation.

1.3 Applications

- Positive selection or depletion of cells expressing the mouse CD140a (PDGFRα) antigen.
- Isolation or depletion of CD140a (PDGFRα) positive oligodendrocyte precursors from dissociated mouse brain tissue.

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1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS).

- The Neural Tissue Dissociation Kit (P) (# 130-092-628) is recommended for the generation of single-cell suspensions of neural cells from mouse brain tissue.

  ▲ **Note:** If additional cell surface epitopes of interest are papain sensitive, the Neural Tissue Dissociation Kit (T) (# 130-093-231) can be used.

- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.

- MACS Columns and MACS Separators: CD140a (PDGFRα)$^+$ cells can be enriched by using MS or LS Columns or depleted with the use of LD Columns. Positive selection or depletion can also be performed by using the autoMACS® Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro

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

- (Optional) Fluorochrome-conjugated Labeling Check Reagents to stain labeled cells for flow cytometric analysis, e.g., Labeling Check Reagent-PE (# 130-095-228). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** The use of CD140a antibodies, clone APA5, is not recommended for analysis of cells that are labeled with CD140a (PDGFR α) MicroBeads.

- (Optional) Human PDGF-AA, research grade (2 µg: 130-093-977; 10 µg: 130-093-978), Mouse FGF-2, research grade (10 µg: 130-105-787; 50 µg: 130-105-786)

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

- (Optional) MACS Neuro Medium (# 130-093-570) and MACS NeuroBrew®-21 (# 130-093-566) for cultivation.

- (Optional) gentleMACS™ Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937) and gentleMACS C Tubes (# 130-093-237, # 130-096-334).

2. Protocol

2.1 Sample preparation

For the preparation of single-cell suspensions from neural tissues refer to the data sheet of the Neural Tissue Dissociation Kit (P) (# 130-092-628), which can be used in combination with the gentleMACS Dissociator (# 130-092-235).

▲ **Note:** If additional cell surface epitopes of interest are papain sensitive, the Neural Tissue Dissociation Kit (T) (# 130-093-231) can be used.

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

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. For best performance it is recommended to use at least 5×10^6 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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) 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 10 µL of FcR Blocking Reagent per 10^7 total cells.
5. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of CD140a (PDGFRα) MicroBeads per 10^7 total cells.
7. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^7 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3 or 2.4).

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2.3 Magnetic separation

▲ Choose an appropriate MACS® Column and MACS Separator according to the number of total cells and the number of CD140a (PDGFRα)$^+$ 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 MS or 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 the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **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 the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of CD140a (PDGFRα)$^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

   ▲ **Note:** Elution of the cells from the column after the separation should be performed with cell culture medium if cells are to be taken directly into culture, otherwise elute with buffer as before.

   ▲ **Note:** Keep handling time of cells in PBS/BSA buffer to a minimum.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the 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 that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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.

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.

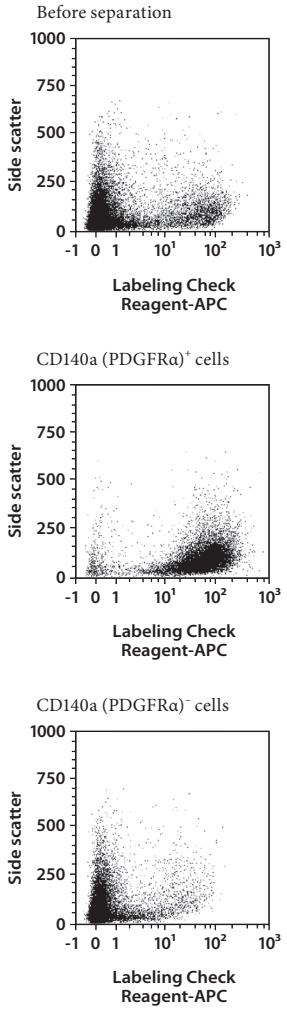
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
4. For a standard separation choose one of the following programs:

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

   Depletion: Depl025
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using the CD140a (PDGFRα) MicroBead Kit

CD140a (PDGFRα)$^+$ cells were isolated from P2 CD-1 mouse brain tissue using the CD140a (PDGFRα) MicroBead Kit, the Neural Tissue Dissociation Kit (P), the gentleMACS™ Dissociator, a MiniMACS Separator, and an MS Column. Cells were fluorecently stained with Labeling Check Reagent-APC 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. Heldin, C.H. and Westermark, B. (1999) Mechanism of action and *in vivo* role of platelet-derived growth factor. Physiol. Rev. 79: 1283–1316.
2. Takakura, N. *et al.* (1997) PDGFRα expression during mouse embryogenesis: immunolocalization analyzed by whole-mount immunohistostaining using the monoclonal antibody anti-mouse PDGFR alpha antibody APA5. J. Histochem. Cytochem. 45(6): 883–893.
3. Sim, F. J. *et al.* (2011) CD140a identifies a population of highly myelinogenic migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. Nat. Biotechnol. 29: 934–941.
4. Nishiyama, A. *et al.* (1996) Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. J. Neurosci. Res. 43: 299–314.
5. Nishiyama, A. *et al.* (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nature reviews, Neurosci. 10: 9–22.

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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