Miltenyi Biotec

Retinal Ganglion Cell Isolation Kit

rat

Order no. 130-096-209

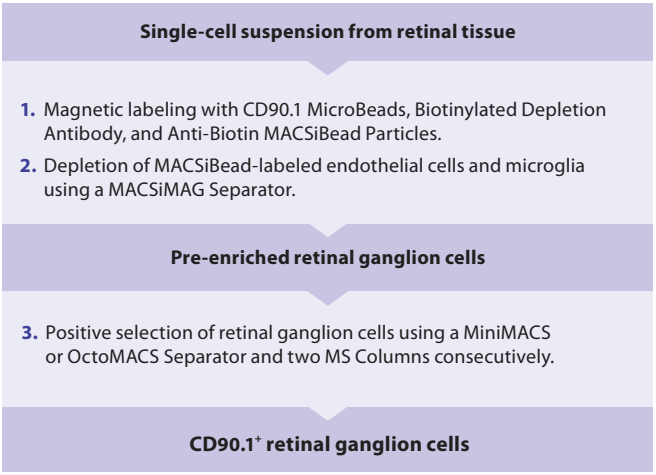
Contents

1. Description

Components	**0.3 mL Biotinylated Depletion Antibody:** Biotin-conjugated monoclonal antibody. **2.5 mL Anti-Biotin MACSiBead™ Particles** **1.25 mL CD90.1 MicroBeads, mouse and rat:** MicroBeads conjugated to monoclonal mouse anti-mouse/rat CD90.1 antibodies (isotype: mouse IgG2a)
Capacity	For 25 separations. The specified number of separations is valid when using 10 retinas (5 rats, age P6–7) per sample.
Product format	All components 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

The retinal ganglion cells are magnetically labeled with CD90.1 MicroBeads. Endothelial cells and microglia, which also express CD90.1, are concurrently labeled with the Biotinylated Depletion Antibody and Anti-Biotin MACSiBead™ Particles. The depletion of the MACSiBead-labeled endothelial cells and microglia is performed using the MACSiMAG™ Separator. The retinal ganglion cells are pre-enriched in the supernatant. The pre-enriched cell suspension is loaded onto a MACS® MS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD90.1+ retinal ganglion cells are retained in the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained CD90.1+ cells are eluted as the positively selected cell fraction, and the positively selected cell fraction is separated over a second column.



1.2 Background information

The Retinal Ganglion Cell Isolation Kit, rat has been developed for the isolation of retinal ganglion cells (RGCs) from rat retina. Retinal ganglion cells are neurons of the central nervous system and project information from the eye to the visual center of the brain. Degeneration of RGCs leads to blindness in ophthalmic diseases like glaucoma. RGCs were among the first neurons to be isolated[1] and cultured under defined conditions[2]. They have been used to study interaction of neurons with glial cells[3,4]. In pre-clinical research, RGCs are useful for the development of neuroregenerative therapies. The best period for isolation of these neurons is postnatal days 6–7. High viability and normal morphology are observed after isolating RGCs using magnetic cell sorting[5].

1.3 Applications

- Isolation of neurons, namely rat retinal ganglion cells.

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140-003-194.02

1.4 Reagent and instrument requirements

- DPBS/BSA buffer: Dulbecco's phosphate-buffered saline (DPBS) with Ca^{2+} and Mg^{2+} and with 0.5% bovine serum albumin (BSA), e.g., 100 mL DPBS and 0.5 g BSA. Store buffer at 2–8 °C for up to 5 days. Bring to room temperature before use.
- MS Columns (# 130-042-201), maximum column capacity: cells derived from 40 retinas (20 rats, age P6–7)
- MiniMACS™ Separation Unit (# 130-042-201) or OctoMACS™ Separation Unit (# 130-042-109)
- 5 mL polystyrene round bottom tubes
- 15 mL polystyrene round bottom tubes
- Neural Tissue Dissociation Kit – Postnatal Neurons (# 130-094-802)
- MACSmix™ Tube Rotator (# 130-090-753)
- MACSiMAG™ Separator (# 130-092-168)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD90.1-FITC, mouse and rat (# 130-094-527) or CD48-PE (Cedarlane Laboratories CL045PE). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) MACSQuant® Analyzer (# 130-092-197)
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

▲ Prepare a single-cell suspension from rat retina tissue using the Neural Tissue Dissociation Kit – Postnatal Neurons (# 130-094-802). For a detailed protocol refer to the data sheet of the Neural Tissue Dissociation Kit – Postnatal Neurons (# 130-094-802).

The Retinal Ganglion Cell Isolation Kit was developed for use in combination with the Neural Tissue Dissociation Kit – Postnatal Neurons) and ensures epitope integrity for the magnetic labeling. The use of other dissociation protocols has not been tested.

2.2 Magnetic labeling

▲ All steps must be performed at room temperature unless otherwise indicated.

▲ Volumes for magnetic labeling given below are for cells from 10 retinas (5 rats, age P6–7) in a final volume of 500 µL. Working with fewer rats is not recommended. When working with retinas from more rats, scale up all reagent volumes and total volumes accordingly, e.g., for 20 retinas from 10 rats, use twice the volume of all indicated reagent volumes and total volumes.

1. Resuspend the cells obtained from 10 retinas (5 rats) in 440 µL of DPBS/BSA buffer.
2. Add 50 µL of CD90.1 MicroBeads and 10 µL of Biotinylated Depletion Antibody.
3. Mix gently and incubate for 15 minutes at room temperature. Mix gently every 5 minutes.

   (Optional) For flow cytometric analysis, add staining antibodies, e.g., 25 µL CD90.1-FITC (# 130-094-527, dilution 1:20) and 3.1 µL CD48-PE (dilution 1:160, Cedarlane Laboratories) after 5 minutes of this incubation period. Mix gently and continue incubation for the remaining 10 minutes.
4. Wash cells by adding 5 mL of DPBS/BSA buffer and centrifuge at 130×g for 5 minutes. Aspirate supernatant carefully.
5. Resuspend in 400 µL of DPBS/BSA buffer by pipetting gently 15 times using a 1 mL pipette tip. Do not vortex the cells.
6. Resuspend Anti-Biotin MACSiBeads Particles thoroughly before use to obtain a homogeneous dispersion of MACSiBead Particles in solution, e.g., by vortexing for 30 seconds. Immediately afterwards pipette 100 µL into a 5 mL tube.
7. Immediately add the 400 µL of single-cell suspension and mix very gently with the Anti-Biotin MACSiBeads Particles.
8. Incubate for 15 minutes at room temperature using the MACSmix Tube Rotator (permanent run, medium speed/8 rpm).

   ▲ **Note:** The large rack of the MACSmix Tube Rotator can be adjusted to the size of the 5 mL tube.
9. Proceed to magnetic separation: Depletion of endothelial cells and microglia (2.3).

2.3 Magnetic separation: Depletion of endothelial cells and microglia

▲ Choose a MACSiMAG Separator.

▲ Do not touch the adhered cells when pipetting off the supernatant.

1. Remove the 5 mL tube from MACSmix Tube Rotator and open it. Rinse lid with 300 µL of DPBS/BSA and add it to the 500 µL in the tube.
2. Place 5 mL tube in the magnetic field of the MACSiMAG Separator.

   ▲ **Note:** Use tube rack to insert tubes from 1.5 mL to 5 mL in size. For details see the MACSiMAG Separator data sheet.
3. Allow the MACSiBead-labeled cells to adhere to the wall of the tube for 3 minutes.
4. Retaining the tube in the MACSiMAG Separator, carefully pipette supernatant into a new 5 mL tube placed outside of the magnet. The supernatant is the pre-enriched fraction containing the retinal ganglion cells.

   ▲ **Note:** In this step, the supernatant does not need to be completely removed; remaining target cells will be collected in the next step.
5. Wash the adhered cells by rinsing the wall of the tube in the MACSiMAG Separator with 0.5 mL of DPBS/BSA.
6. Allow the cells to adhere to the tube wall for one minute.
7. Retaining the tube in the MACSiMAG Separator, carefully pipette supernatant into the tube containing the pre-enriched fraction of step 4.
8. Place 5 mL tube containing the pre-enriched fraction of step 7 into the MACSiMAG Separator. Allow remaining MACSiBead-labeled cells to adhere to the tube wall for 3 minutes.
9. Retaining the tube in the MACSiMAG Separator, carefully pipette the supernatant (pre-enriched fraction) into a 15 mL tube.
10. Centrifuge at 130×g for 2 minutes at room temperature. Aspirate supernatant carefully.
11. Resuspend cells carefully in 500 µL of DPBS/BSA buffer by pipetting gently 15 times using a 1 mL pipette tip. Do not vortex the cells.
12. Proceed to magnetic separation: Positive selection of $CD90.1^+$ retinal ganglion cells (2.4).

2.4 Magnetic separation: Positive selection of $CD90.1^+$ retinal ganglion cells

▲ Choose an appropriate MACS Separator. For details see section 1.4.

▲ A maximum of cells derived from up to 40 retinas or 20 rats (age P6–7) can be applied to an MS Column.

▲ Samples are separated using 2 MS Columns consecutively.

▲ Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

1. Place 2 MS Columns in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
2. Prepare columns by rinsing with 500 µL of DPBS/BSA buffer.
3. Apply cell suspension onto the first MS Column. Collect flow-through containing unlabeled cells in case analysis is of interest.
4. Wash column with 3×500 µL of DPBS/BSA buffer. Collect unlabeled cells that pass through and combine with flow-through from step 3 for optional analysis.
5. Remove first column from the separator and hold it over the second equilibrated MS Column. Pipette 1 mL of DPBS/BSA buffer onto the first MS Column and immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
6. Let cells pass through the second MS Column and wash with 3×500 µL of DPBS/BSA buffer.
7. Remove second MS Column from the separator and place it onto a suitable collection tube.
8. Pipette 1 mL of DPBS/BSA buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
9. Determine cell number by using, for example, the MACSQuant Analyzer. Cultivate cells in a poly-L-lysine-coated dish in an appropriate density at 37 °C or proceed immediately to subsequent applications.

   ▲ **Note:** Unbound MACSiBead Particles may be observed in cell culture but will not harm the cells.

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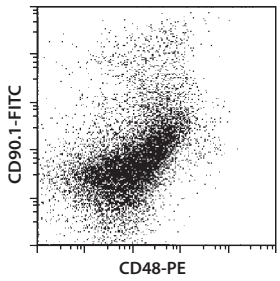
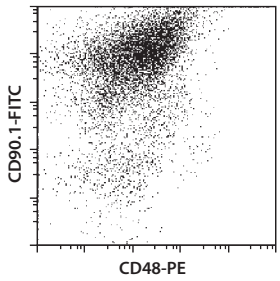
3. Example of a separation using the Retinal Ganglion Cell Isolation Kit

Isolation of CD90.1$^+$ retinal ganglion cells from postnatal day 7 rat retina tissue using the Neural Tissue Dissociation Kit - Postnatal Neurons, the Retinal Ganglion Cell Isolation Kit, a MACSiMAG Separator, an OctoMACS Separator, and MS Columns. Cells were fluorescently stained with CD48-PE and CD90.1-FITC (# 130-094-527) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation



CD90.1$^+$ retinal ganglion cells



4. References

1. Barres, B. A. *et al.* (1988) Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning.Neuron 1: 791–803.
2. Meyer-Franke, A. *et al.* (1995) Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron 15: 805–819.
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4. Göritz, C. *et al.* (2007) Glia-induced neuronal differentiation by transcriptional regulation. Glia 55: 1108–1122.
5. Pennartz, S *et al.* (2010) Purification of retinal ganglion cells. MACS&more Vol. 12-2/2010.
6. Bottenstein, J. E. and Sato, G. H. (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc. Natl. Acad. Sci. U.S.A. 76: 514–517.

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.

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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