Miltenyi Biotec

Neuron Isolation Kit
mouse

2× 1 mL	130-115-389
2× 100 µL	130-115-390

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	**1 mL Non-Neuronal Cell Biotin-Antibody Cocktail, mouse:** monoclonal antibodies conjugated to biotin against non-neuronal cells. **1 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1). or **100 µL Non-Neuronal Cell Biotin-Antibody Cocktail, mouse:** monoclonal antibodies conjugated to biotin against non-neuronal cells. **100 µL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	2× 1 mL for 5×10^8 total cells, up to 50 separations or 2× 100 µL for 5×10^7 total cells, up to 5 separations.
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

Using the Neuron Isolation Kit, mouse neuronal cells are isolated by depletion of non-neuronal cells. Non-neuronal cells are magnetically labeled with biotin-conjugated monoclonal antibodies specific for non-neuronal cells followed by anti-biotin monoclonal antibodies conjugated to MicroBeads. The magnetically labeled non-neuronal cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled neuronal cells run through.

1.2 Background information

The Neuron Isolation Kit, mouse is an indirect magnetic labeling system for the isolation of untouched neurons from cell suspensions of mouse neural tissue. Non-neuronal cells like astrocytes, oligodendrocytes, microglia, endothelial cells, fibroblasts, except erythrocytes, are indirectly magnetically labeled by using biotin-conjugated antibodies specific for non-neuronal cells in combination with Anti-Biotin MicroBeads. Isolation of highly pure unlabeled neuronal cells is achieved by depletion of the magnetically labeled cells.

The cell number and composition of the neuronal cell fraction differs according to the mouse age and the brain region used for cell isolation.

The isolation of neurons has been tested with CD-1® mice aged from embryonic day 18 (E18) to adult. For optimal results, the Neural Tissue Dissociation Kit – Postnatal Neurons or the Adult Brain Dissociation Kit have been used prior to this kit.

1.3 Applications

- Untouched isolation of mouse neuronal cells from neural cell suspensions of neonatal and adult mice.

1.4 Reagent and instrument requirements

- DPBS/BSA buffer: Prepare a solution containing Dulbecco's phosphate-buffered saline (DPBS) with Ca^{2+} and Mg^{2+} and 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with DPBS. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
  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.

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

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- MACS Columns and MACS Separators: Depletion of non-neuronal cells can be performed on an LS Column. Depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS
autoMACS	5×10^7	10^8	autoMACS Pro

- Neural Tissue Dissociation Kit – Postnatal Neurons (# 130-094-802) for the generation of single-cell suspension from mouse brain tissue up to P7.
- Adult Brain Dissociation Kit, mouse and rat (# 130-107-677) for the generation of single-cell suspension from mouse brain tissue older than P7.
- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACS® Neuro Medium (# 130-093-570) and MACS NeuroBrew®-21 (# 130-093-566) for the cultivation of neuronal cells.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-ACSA-2-PE, Anti-O4-PE, or CD11b-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from neural tissues from mouse younger than P7 use the Neural Tissue Dissociation Kit – Postnatal Neurons or for mouse tissue older than P7 the Adult Brain Dissociation Kit, mouse and rat, which may be used in combination with the gentleMACS Dissociators.
As erythrocyte lysis is not included in the Neural Tissue Dissociation Kit - Postnatal Neurons, a red blood cell lysis can be performed by using Red Blood Cell Lysis Solution (10×) (for details refer to Adult Brain Dissociation Kit data sheet, 2.4 Red blood cell removal).

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. Add 80 µL of buffer per 10^7 total cells to the cell pellet.
4. Add 20 µL of the Non-Neuronal Cell Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well. Do not vortex. Incubate for 5 minutes in the refrigerator (2–8 °C).

   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
6. Wash cells by adding 1 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add 80 µL of buffer per 10^7 total cells to the cell pellet.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).

   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.
10. Adjust volume to 500 µL for up to 10^7 cells with buffer. Do not centrifuge!

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

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2.3 Magnetic separation

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

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the LS 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 2×1 mL of buffer. Collect unlabeled cells (neuronal 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. (Optional) Remove LS Column from the separator and place it on a suitable collection tube.
6. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Use DPBS/BSA buffer for magnetic separation with the autoMACS Pro Separator. Buffers used for operating the autoMACS Pro 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.

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 following program:

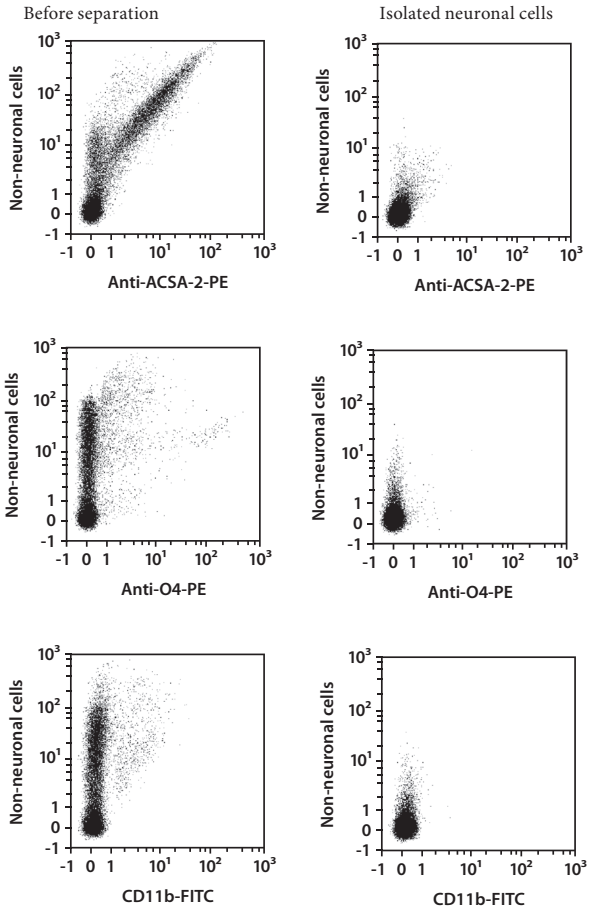
   Depletion: Depl05

   Collect negative fraction in row B of the tube rack.

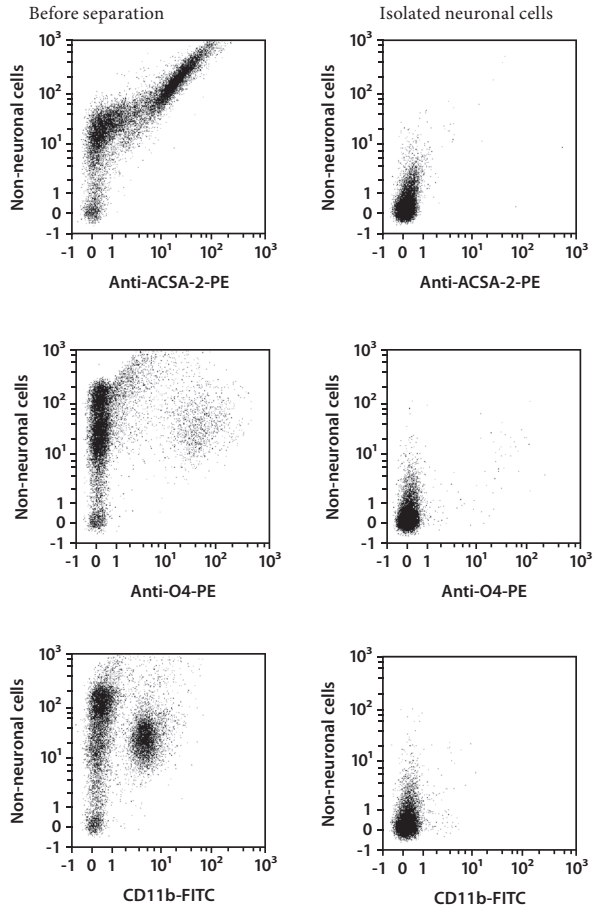
3. Example of a separation using the Neuron Isolation Kit

Neuronal cells were isolated from P2 and from 9 weeks old CD-1® mouse brains. P2 mouse brain was dissociated using the Neural Tissue Dissociation Kit – Postnatal Neurons and adult mouse brain using the Adult Brain Dissociation Kit, mouse and rat. The neurons were isolated using the Neuron Isolation Kit, mouse, LS Columns, and a MidiMACS™ Separator.
Cells were fluorescently stained with an antibody cocktail specific for non-neuronal cells and the astrocyte-specific antibody Anti-ACSA-2-PE, the oligodendrocyte-specific antibody Anti-O4-PE, and the microglia-specific antibody CD11b-FITC. Cells were analyzed by flow cytometry using the MACSQuant™ Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.
For further results and information refer to www.miltenyibiotec.com/130-115-389.

A) Neonatal cells



B) Adult cells



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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