Miltenyi Biotec

Adult Neural Stem Cell Analysis Cocktail Kit, anti-mouse

Order no. 130-121-268

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	**250 µL Adult Neural Stem Cell Analysis Cocktail, anti-mouse** containing:
	GLAST (ACSA-1) Antibody, anti-human/mouse/rat, APC (clone: ACSA-1, isotype: mouse IgG2aκ)
	Plexin-B2 Antibody, anti-mouse, PE, REAfinity™ (clone: REA445, isotype: recombinant human IgG1)
	CD24 Antibody, anti-mouse, VioBlue®, REAfinity (clone: REA743, isotype: recombinant human IgG1)
	CD45 Antibody, anti-mouse, VioBlue, REAfinity (clone: REA737, isotype: recombinant human IgG1)
	Ter-119 Antibody, anti-mouse, VioBlue, REAfinity (clone: REA847, isotype: recombinant human IgG1)
	250 µL FcR Blocking Reagent, mouse
Capacity	25 tests or up to 2.5×10^7 total cells.
Product format	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 Background information

Neural stem cells (NSCs) in the adult subventricular zone (SVZ) and the dentate gyrus (DG) have the capacity to self-renew and generate new neural cells throughout lifetime. Their ability to react to brain injury by generating new neural cells makes them a valuable cell source for endogenous repair in the adult brain. NSCs are a very rare and sensitive cell population. A complex marker combination is necessary to distinguish them from other cells.

The Adult Neural Stem Cell Analysis Cocktail Kit, anti-mouse enables a reliable identification of NSCs from the SVZ of mouse brain tissue for subsequent sorting and analysis without requirement of transgenic mice. The cocktail includes two NSC-specific antibodies (GLAST and Plexin-B2 antibodies) and three exclusion antibodies to exclude erythrocytes, leukocytes, microglia, neurons, ependymal cells, and neuroblasts.

NSCs can be sorted using the MACSQuant® Tyto® cell sorter and analyzed by flow cytometry using the MACSQuant Analyzer 10. The sorted cells are ready for different downstream applications.

The kit applies recombinant engineered REAfinity Antibodies. REAfinity Antibodies are recombinant antibodies that provide superior lot-to-lot consistency and purity compared to mouse or rat hybridoma-derived, monoclonal antibodies. They have been recombinantly engineered to produce highly specific antibodies that require no FcR blocking step. Additionally, they all have the same IgG1 isotype, requiring less isotype controls.

1.2 Applications

- Identification and enumeration of NSCs from adult mouse SVZ for subsequent sorting and analysis.

1.3 Reagent and instrument requirements

- Flow cytometer equipped with a red (640 nm), a blue (488 nm), and a violet (405 nm) laser, e.g., MACSQuant Analyzer 10 (# 130-096-343), MACSQuant Analyzer 16 (# 130-109-803), or a cell sorter, e.g., MACSQuant Tyto (# 130-103-931).
- MACSQuant Tyto Running Buffer (# 130-107-206, # 130-107-207).
- (Optional) 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).

  ▲ **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 human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca2+ or Mg2+ are not recommended for use.
- (Optional) Propidium Iodide Solution (# 130-093-233) for detection of dead cells during flow cytometric analysis.
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) or anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

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140-005-510.03

2. Protocol

▲ For a complete protocol refer to the application protocol "Isolation and cultivation of adult neural stem cells from adult mouse brain" at www.miltenyibiotec.com/applications.

▲ 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.

▲ 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.

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

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 MACSQuant Tyto Running Buffer.
4. Add 10 µL of the FcR Blocking Reagent, mouse.
5. Add 10 µL of Adult Neural Stem Cell Analysis Cocktail, anti-mouse.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1 mL of MACSQuant Tyto Running Buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^6 cells in 1 mL of MACSQuant Tyto Running Buffer.

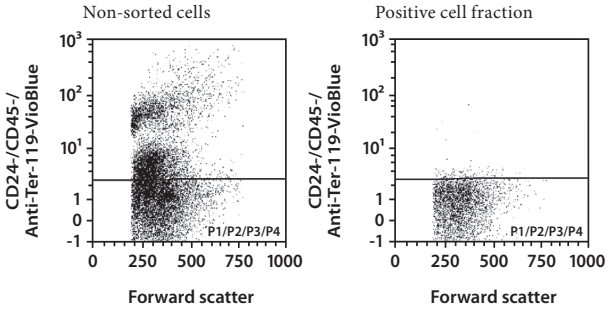
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
9. Process immediately to sorting or analysis of NSCs.

3. Example of immunofluorescent staining with the Adult Neural Stem Cell Analysis Cocktail Kit, anti-mouse

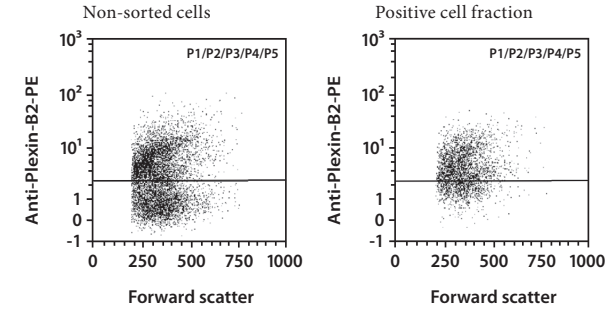
SVZ tissue from adult mice (7–9 weeks) was dissociated using the Neural Tissue Dissociation Kit (T) (# 130-093-231) and the gentleMACS™ Octo Dissociator with Heaters (# 130-096-427). After dissociation, cell debris was removed using the Debris Removal Solution. Subsequently, cells were treated with FcR Blocking Reagent, mouse and stained with the Adult Neural Stem Cell Analysis Cocktail, anti-mouse. NSCs were then sorted using the MACSQuant Tyto. Samples of non-sorted cells as well as the positive cell fraction were analyzed by flow cytometry using the MACSQuant Analyzer 10.

For identification of NSCs, a hierarchical gating strategy was applied. First, cell debris, doublets, and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. After exclusion of cells labeled by CD24, CD45, and Ter-119 antibodies (A), a gate was set on plexin-B2$^+$ cells (B), before plexin-B2$^+$GLAST$^+$ NSCs were identified (C).

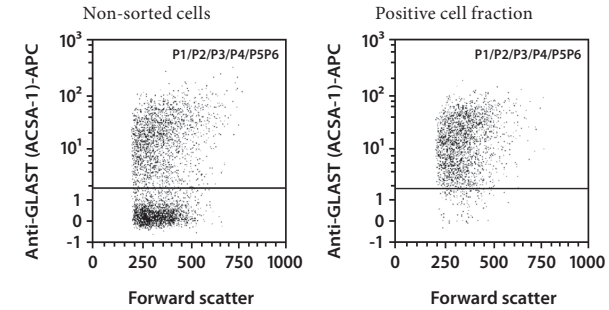
A)



B)



C)



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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