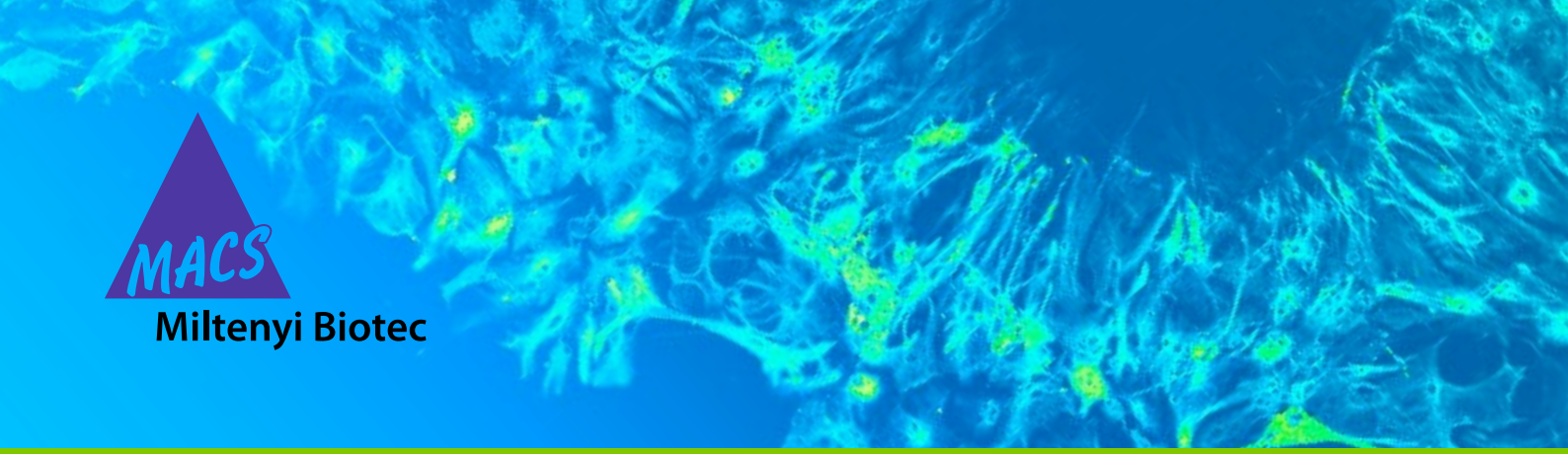

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Sorting and cultivation of adult neural stem cells from mice

Introduction

Neural stem cells (NSCs) are able to self-renew and to differentiate into various neural cells. Residing mainly in the adult subventricular zone (SVZ) and the dentate gyrus, they are a valuable tool to react to brain injury and degeneration by generating new neurons throughout their lifetime. However, NSCs are a very rare and sensitive cell population that is defined by a complex marker combination, which makes them difficult to isolate and to purify.

Here we show a new approach to purifying NSCs from the SVZ of wild type mouse brain. We optimized and automated the tissue dissociation and cell sorting process to isolate viable single NSCs with preserved epitopes. The Neural Tissue Dissociation Kit (T) together with the gentleMACS™ Octo Dissociator with Heaters dissociates mouse SVZ tissue gently and efficiently. The novel antibody cocktail included in the Adult Neural Stem Cell Sorting and Analysis Kit, mouse detects exclusion and NSC-specific markers for unambiguous and reliable isolation of pure NSCs. The MACSQuant® Tyto® Sorter, a novel multiparameter cell sorting device, identifies and sorts NSCs. In contrast to conventional droplet sorters, the MACSQuant Tyto sorts cell suspensions via a microchip-based technology that guarantees sterile and gentle cell isolation. Subsequent cell cultivation with MACS® Neuro Medium and MACS NeuroBrew®-21 Supplement facilitates differentiation into neurons, astrocytes, or oligodendrocytes and enhances the ability of NSCs to remain in an undifferentiated state.

The combination of optimized sample preparation, sophisticated cell sorting technology, and compatible cell cultivation conditions is the perfect tool for NSC isolation, characterization, and functional analysis.

Materials and methods

Dissociation, identification, and sorting of NSCs from the SVZ of mice

Brains from adult mice (7–9 weeks old) were dissociated with the Neural Tissue Dissociation Kit (T) and the gentleMACS™ Octo Dissociator with Heaters. Debris was removed with the Debris Removal Solution and cells were labeled with an antibody cocktail staining exclusion and NSC-specific markers (tab. 1).

Markers for identification of NSCs	Marker expression on NSCs	Antibodies
NSC-specific markers		
GLAST (ACSA-1)	High	Anti-GLAST (ACSA-1)-APC
plexin-B2	Mid to high	Anti-Plexin-B2-PE
NSC exclusion markers		
CD24	Negative	CD24-VioBlue®
CD45	Negative	CD45-VioBlue
Ter-119	Negative	Anti-Ter-119-VioBlue

Table 1: Marker expression and antibodies for identification of NSCs from mouse brain. Antibody cocktail is included in the Adult Neural Stem Cell Sorting and Analysis Kit, mouse.

The gating strategy was established with the MACSQuant® Analyzer 10 and is depicted for a non-sorted cell sample in figure 1. First, debris as well as doublets and dead cells were excluded (fig. 1A–C). Cells positive for the exclusion markers CD24, CD45, and Ter-119 were gated out (fig. 1D) and remaining cell subsets were gated for plexin-B2 (fig. 1E) and GLAST (fig. 1F) expression.

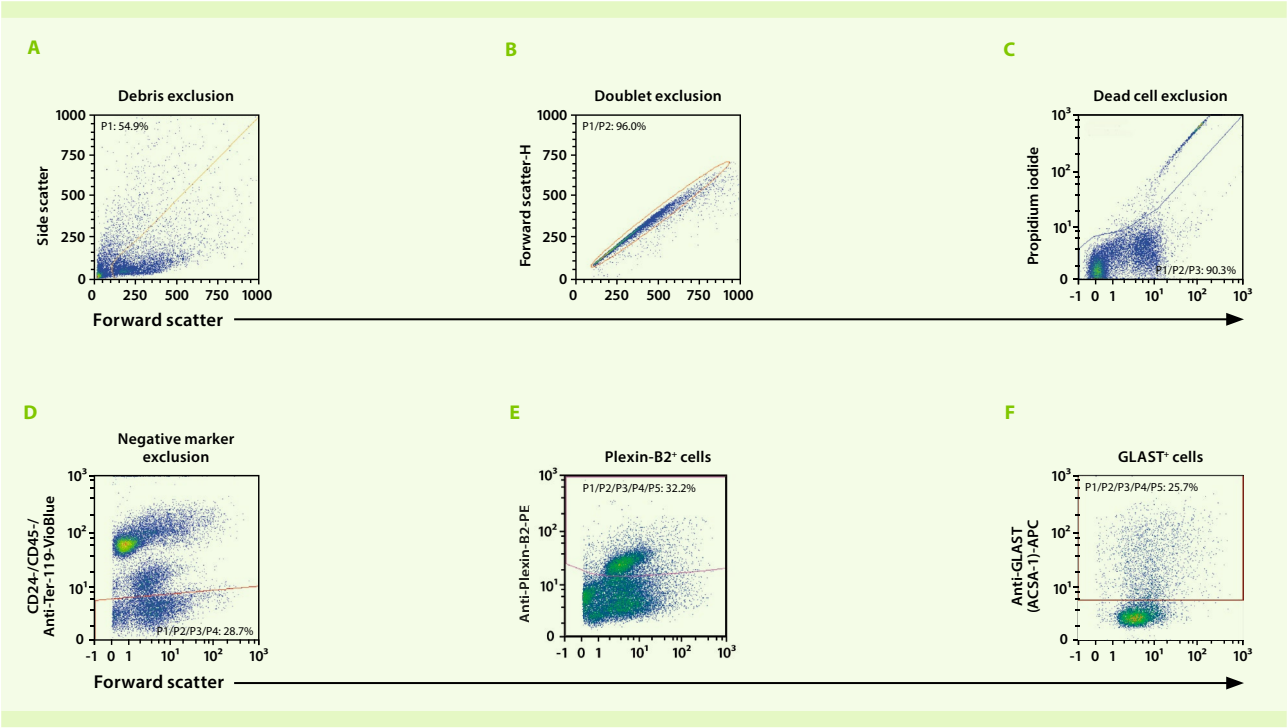

Figure 1: Gating strategy to isolate NSCs. Cells from the SVZ from adult mice were analyzed on the MACSQuant Analyzer 10. First, debris (A), doublets (B), and dead cells (C) were excluded from analysis. After exclusion of cells positive for CD24, CD45, and Ter-119 (D), a gate was set on plexin-B2+ cells (E), before plexin-B2+GLAST+ NSCs were identified (F).

The gating strategy was applied to the microchip-based flow sorter MACSQuant® Tyto®. Cells were sorted at 4.3 mL/h and a pressure of 100–150 mbar. Sorting of NSCs derived from SVZ tissues of five adult mice was carried out according to the gating strategy depicted in figure 1 within 30 min. Accordingly, the whole NSC isolation process including dissection of the SVZ, tissue dissociation, antibody staining, and cell sorting takes approximately 3 hours (tab. 2).

Processing step	Time
Dissection of 10 SVZ	60 min
Dissociation procedure	70 min
Antibody staining	20 min
Sorting on MACSQuant Tyto	30 min
Total time	**3 h**

Table 2: The established workflow facilitates NSC isolation within 3 hours.

Neurosphere assay and neural differentiation assay

Isolated cells were cultivated on ultra-low attachment plates in MACS® Neuro Medium supplemented with MACS NeuroBrew®-21, EGF (20 ng/mL), FGF-2 (20 ng/mL) for 7–10 days. To assess the self-renewal capacity, generation of secondary neurospheres was tested. To that end, primary neurospheres were dissociated using the Neurosphere Dissociation Kit (P) and plated again for 7–10 days in the same medium.

For differentiation, neurospheres were transferred to poly-L-lysine–coated 24-well glass bottom plates in MACS Neuro Medium supplemented with MACS NeuroBrew-21. After 7 days of differentiation, cells were fixed and subjected to immunocytochemical analysis using antibodies specific for the astrocyte markers GFAP, nestin, and GLAST, the neuronal cell marker MAP2, and the oligodendrocyte marker O4.

Results

New dissociation and sorting procedures enable gentle fluorescence-based sorting of neural stem cells from mouse SVZ

Dissociation of SVZ from one mouse yielded $3.7\times10^5 \pm 9.3\times10^4$ cells with a viability rate of >97% (n = 12). Subsequently, cells were sorted with the MACSQuant® Tyto® Sorter. To examine purity, viability, and yield of NSCs sorted with this workflow, non-sorted cells as well as samples from the positive and negative cell fractions were analyzed by flow cytometry (fig. 2). The applied sorting procedure significantly enriched plexin-B2+GLAST+ cells. Sorting of $3.7\times10^5 \pm 9.3\times10^4$ total cells resulted in $3.6\times10^4 \pm 8.0\times10^3$ plexin-B2+GLAST+ NSCs with a purity of >95% and a viability rate of >93% as analyzed by PI staining (data not shown, n = 6).

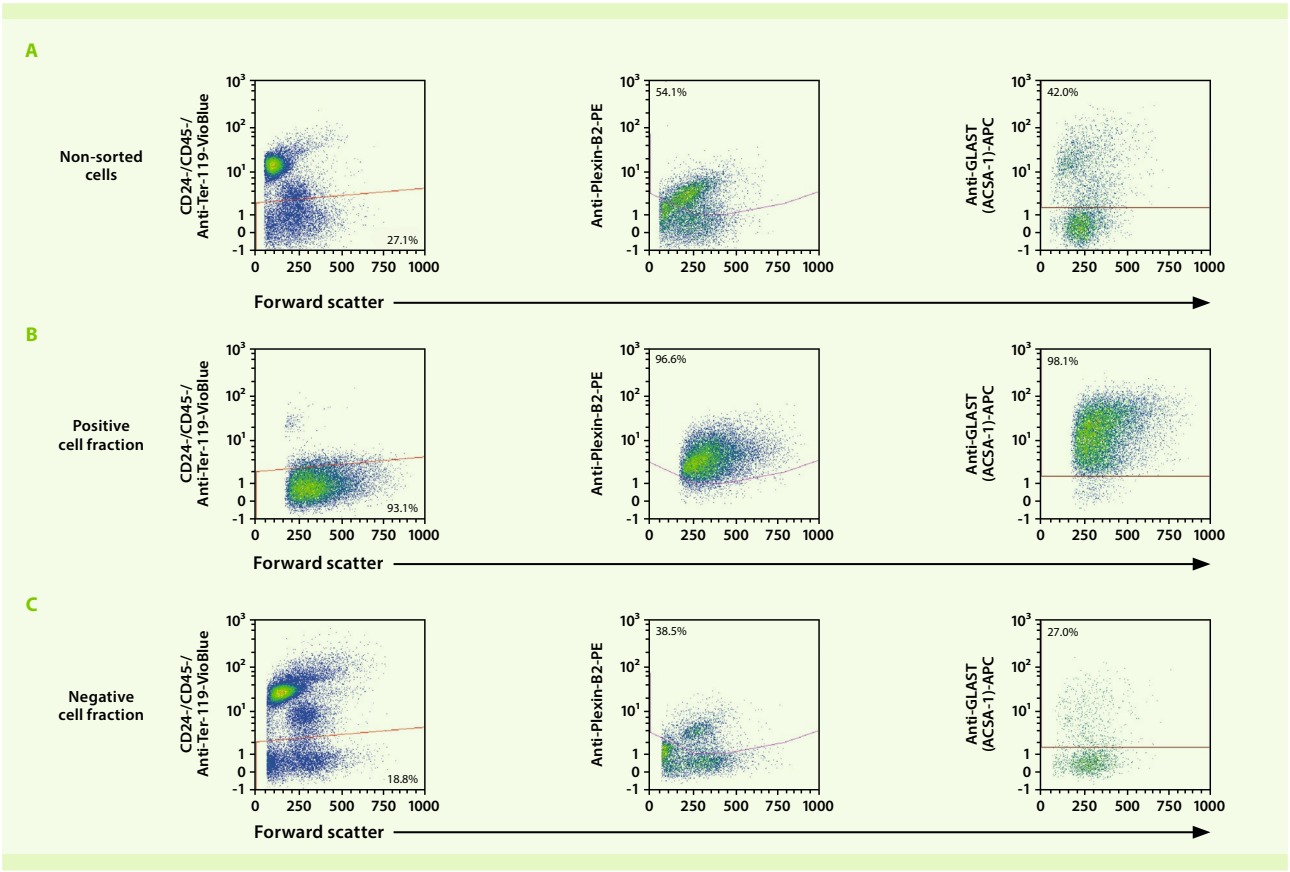

Figure 2: Sorting of NSCs using the antibody cocktail (table 2) and the MACSQuant® Tyto® Sorter substantially enriches plexin-B2⁺GLAST⁺ NSCs. Analysis of non-sorted cells (A) and cells from the positive (B) and negative (C) fractions that had been sorted on the MACSQuant Tyto according to the gating strategy depicted in figure 1.

Isolated NSCs give rise to primary and secondary neurospheres, and differentiate into glial cells and neurons

Neurosphere assays are widely used to identify and to quantitate NSCs in a heterogeneous cell population based on their ability to self-renew and to differentiate. To assess the self-renewal capacity of isolated cells, generation of secondary neurospheres was tested. Primary neurospheres were dissociated and plated again for 7–10 days on non-coated plates. Cultivation under these assay conditions led to the formation of a large number of secondary neurospheres (fig. 3A, B).

For differentiation, neurospheres were plated on coated plates and cultured for seven days. Subsequently, cells were fixed and subjected to immunocytochemical analysis using antibodies for the astrocyte markers GFAP, nestin, and GLAST, the neuronal cell marker MAP2, and the oligodendrocyte marker O4. Neurospheres differentiated into glial cells as well as neurons as shown by expression of the markers tested (fig. 3C–F).

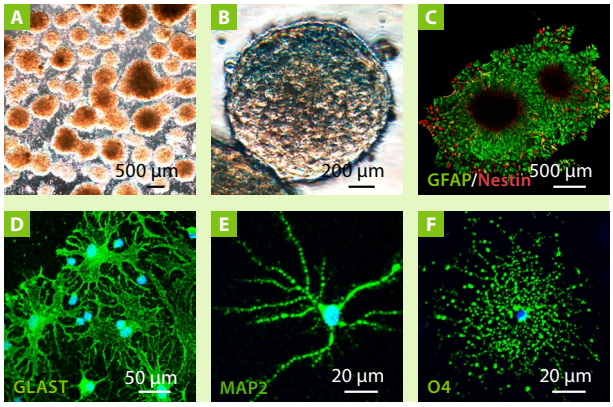

Figure 3: Neurosphere assay to analyze self-renewal and differentiation capacities of isolated NSCs. Cultivation of isolated NSCs under the respective assay conditions led to formation of neurospheres, which gave rise to secondary neurospheres (A–B). Neurospheres differentiated into glial cells as well as neurons as shown by expression of GFAP, nestin, GLAST, MAP2, and O4 (C–F).

Conclusion

Here, we present an adult neural stem cell workflow including SVZ tissue dissociation using the Neural Tissue Dissociation Kit (T) together with the gentleMACS™ Octo Dissociator with Heaters, sorting of NSCs on the MACSQuant® Tyto® Sorter using the novel antibody cocktail included in the Adult Neural Stem Cell Sorting and Analysis Kit, mouse, and cultivation of NSCs using MACS® Neuro Medium and MACS NeuroBrew®-21 supplement.

With this workflow, highly pure and viable NSCs can be obtained in only three hours. The gentle sorting technology of the MACSQuant Tyto allows for a purity of >95% and a viability of >93%. The isolated NSCs form neurospheres in culture and are able to differentiate into neurons and glial cells.

Ordering information

Product	Order no.
Neural Tissue Dissociation Kit (T)	130-093-231
gentleMACS Octo Dissociator with Heaters	130-096-427
Debris Removal Solution	130-109-398
Adult Neural Stem Cell Sorting and Analysis Kit, mouse	130-121-268
MACSQuant Tyto	130-103-931
MACSQuant Analyzer 10	130-096-343
MACS Neuro Medium	130-093-570
MACS NeuroBrew-21	130-093-566
MACS NeuroBrew-21 w/o Vitamin A	130-097-263
Neurosphere Dissociation Kit (P)	130-095-943
Mouse EGF, research grade	130-094-036
Mouse FGF-2, research grade	130-105-787

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