# A novel approach for gentle sorting of adult neural stem cells from wild type mouse brain using the MACSQuant® Tyto®

Carolina Wittwer, Jens Gaiser, Andreas Bosio, and Melanie Jungblut
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

## Introduction

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

Here we show a new approach to purify NSCs from the SVZ of wild type mouse brain. An optimized automated tissue dissociation protocol ensured a high viability rate within the resulting single-cell suspension and a maintained integrity of cell surface marker epitopes. NSCs were identified by detection of the exclusion markers CD24, Ter-119, and CD45 and the NSC-specific markers GLAST and plexin-B2.

Subsequently, purification of NSCs was carried out using the MACSQuant® Tyto®, a novel multiparametric cell sorting device that uses a microchip-based technology for sterile and gentle cell isolation. Neurosphere assays showed that the isolated NSCs formed a large number of neurospheres, which gave rise to secondary neurospheres and differentiated into different neural cell types.

In summary, we present a novel approach for isolation of NSCs from the SVZ that combines optimized cell preparation methods and a sophisticated cell sorting technology based on specific markers.

## Methods

### 1 Experimental setup for isolation of neural stem cells

Figure 1 gives an overview of the experimental setup. The Neural Tissue Dissociation Kit (T) and the gentleMACS™ Octo Dissociator with Heaters enabled the gentle and efficient dissociation of mouse SVZ tissue. This automated process relies on an optimized dissociation program for small tissue samples.

A new protocol for effective removal of cell debris was used to increase the percentage of intact neural cells. This ultimately resulted in a significantly shorter processing time during subsequent flow sorting. Dissociation of SVZ tissue derived from one mouse yielded $3.69\times10^5 \pm 9.3\times10^4$ cells with a viability rate of >97% (n = 12). Subsequently, cells were labeled with an antibody cocktail that allowed for unambiguous flow cytometric identification and sorting of NSCs. Antigens included GLAST and plexin-B2 as positive markers and CD24, Ter-119, and CD45 as negative markers to exclude erythrocytes, leukocytes, microglia, neurons, ependymal cells, and neuroblasts[1] (table 1). The corresponding gating strategy is described in methods section 2. Purification of NSCs was carried out on the microchip-based microfluidic flow sorter MACSQuant Tyto (see methods section 3).

Re-analysis of isolated NSCs was again performed by flow cytometry. In addition, a neurosphere assay was carried out to analyze the capacity of isolated cells for self-renewal and subsequent differentiation (for details refer to results section 2).

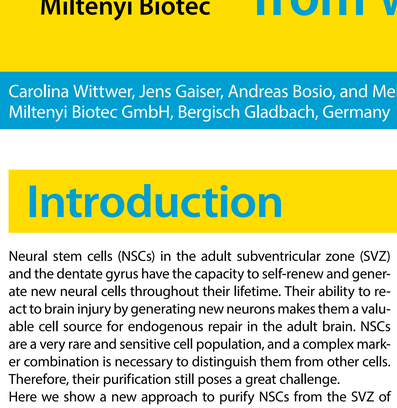


Figure 1

| Antibodies for identification and sorting of neural stem cells | Marker expression on neural stem cells |
|---|---|
| Anti-GLAST (ACSA-1)-APC | High |
| Anti-Plexin-B2-PE | Mid to high |
| CD24-VioBlue® | Negative |
| CD45-VioBlue | Negative |
| Anti-Ter-119-VioBlue | Negative |

Table 1

### 2 Gating strategy for identification of neural stem cells

After dissociating the tissue and clearing the single-cell suspension from debris, cells were stained with the antibody cocktail specified in table 1. For identification of NSCs, a hierarchical gating strategy was designed using the MACSQuant Analyzer 10 and the MACSQuantify Software. First, debris was excluded in the forward scatter versus side scatter plot (fig. 2A). Subsequently, doublets and dead cells were gated out (fig. 2B,C). After exclusion of cells labeled by the negative markers CD24, CD45, and Ter-119 (fig. 2D), a gate was set on plexin-B2$^+$ cells (fig. 2E), before plexin-B2$^+$GLAST$^+$ NSCs were identified (fig. 2F).

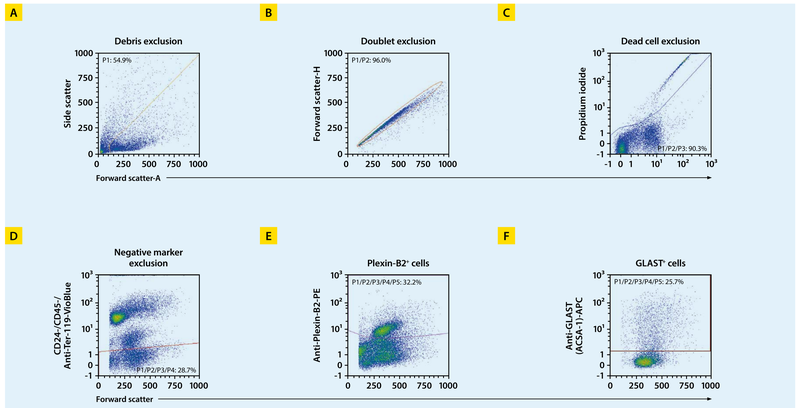


Figure 2

### 3 Flow sorting of neural stem cells using the MACSQuant® Tyto®

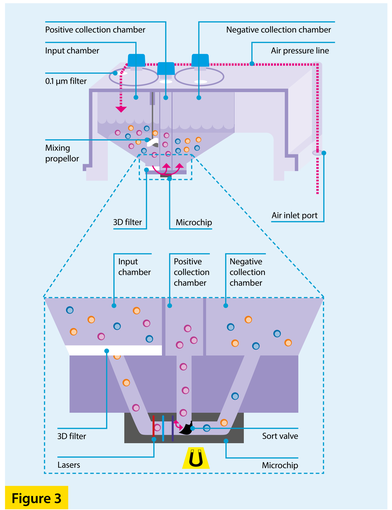


Figure 3

The entire cell sorting process of the MACSQuant Tyto occurs within the closed environment of the MACSQuant Tyto Cartridge (fig. 3). This single-use cartridge contains a sorting microchip, which allows for completely aseptic conditions without cross-contamination between samples. Unlike in conventional droplet sorters, cells do not experience high pressures and no charge is applied, ensuring gentle sorting conditions for high cell viability and maintained functionality. The MACSQuant Tyto allows for high-speed fluorescence-based cell sorting, enabling gentle isolation of NSCs. Cells were sorted at 4.3 mL/h and a pressure of 100–150 mbar. Sorting of NSCs from SVZ tissue derived from five mice was carried out according to the gating strategy depicted in figure 2 and took around 30 min to complete. Accordingly, the whole process including dissection of the SVZ, tissue dissociation, antibody staining, and cell sorting took approximately 3 h (table 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

## Results

### 1 Microchip technology of the MACSQuant® Tyto® enables gentle fluorescence-based sorting of neural stem cells

Upon completion of the sorting process, samples of the non-sorted cells as well as the positive and negative cell fractions were analyzed by flow cytometry to determine purity, viability, and yield according to the gating strategy described above. Figure 4 shows a representative analysis of the different fractions after exclusion of debris, doublets, and dead cells. Sorting of $3.69\times10^5 \pm 9.3\times10^4$ total cells resulted in 36,000 ± 8,000 GLAST$^+$plexin-B2$^+$ NSCs with a purity of >95% and a viability rate of >93% as analyzed by PI staining (n = 6).

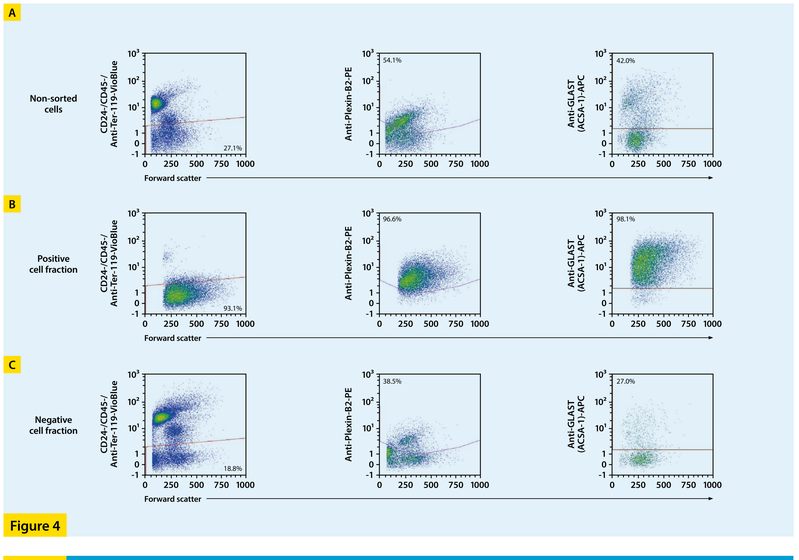


Figure 4

### 2 Isolated neural stem cells give rise to primary and secondary neurospheres and differentiate into glial cells and neurons

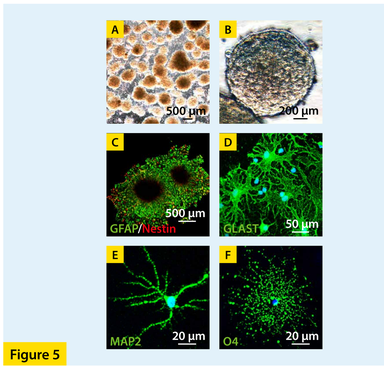


Figure 5

Neurosphere assays are widely used to identify stem cells based on their capacity for self-renewal and their ability to differentiate. Isolated cells were cultivated on ultra-low attachment plates in MACS® Neuro Medium supplemented with MACS NeuroBrew®-21, EGF (20 ng/mL), and 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 plated on poly-L-lysine–coated 24-well glass bottom plates in MACS Neuro Medium supplemented with MACS NeuroBrew-21. After 7 days of culture, 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.

Cultivation of isolated NSCs under the specified assay conditions led to formation of a large number of neurospheres (fig. 5A,B), which gave rise to secondary neurospheres. Furthermore, neurospheres differentiated into glial cells as well as neurons as shown by expression of GFAP, nestin, GLAST, MAP2, and O4 (fig. 5C–F).

## Summary and outlook

- We present a novel approach for isolation of highly pure NSCs from the SVZ of wild type mice based on the positive markers GLAST and plexin-B2 and the negative markers CD24, Ter-119, and CD45.
- The gentle sorting procedure of the MACSQuant Tyto allows for enrichment of NSCs to a purity of >95% and a viability of >93% and maintains functionality of isolated NSCs for further functional and molecular studies.
- The experimental procedure including tissue dissection and dissociation as well as sorting of NSCs on the MACSQuant Tyto can be performed in only 3 h.
- Capacity of the isolated cells for self-renewal and differentiation was tested, and it was shown that the NSCs formed a large number of primary neurospheres, which gave rise to secondary neurospheres and differentiated into neurons as well as different glial cells.

**Literature**
1. Mich, J.K. *et al.* (2014) eLife 3: e02669. doi: 10.7554/eLife.02669.