# A novel automated dissociation procedure allows efficient immunomagnetic isolation of astrocytes, oligodendrocytes, and neurons from adult rodent brain tissue

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## Introduction

Careful tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris is the prerequisite for reliable cellular analysis, cell culture, and cell separation.
In neurobiology, single-cell suspensions are often prepared from embryonic or neonatal rodent neural tissue as neural cells are not yet fully integrated into the neural network and tissue dissociation is relatively easy. In contrast, dissociation of adult brain is very demanding and requires sophisticated mechanical and enzymatic treatment to degrade the extracellular matrix and successfully disaggregate the tightly connected neural cells.
We have developed a new automated method for gentle dissociation of adult rodent brain tissue by combining mechanical dissociation using the gentleMACS™ Octo Dissociator with an optimized enzymatic treatment. Dissociation is followed by a novel procedure for removal of debris and erythrocytes, which is crucial for effective cell isolation. The standardized process allows fast and reproducible dissociation of adult rodent brain tissue and was optimized to increase the number of viable cells.
Protocols for the magnetic isolation (MACS® Technology) of astrocytes, oligodendrocytes, and neurons to high purities were also established and cultivation conditions were carefully optimized for each cell type.
Taken together, we developed a standardized process that allows gentle automated dissociation of adult rodent brain tissue and convenient magnetic isolation as well as cultivation of highly viable astrocytes, oligodendrocytes, and neurons.

## Results

### 1 Automated dissociation of adult mouse brain tissue

We developed a novel process for the automated dissociation of adult rodent brain tissue by combining an optimized enzymatic treatment with gentle mechanical dissociation using the gentleMACS Octo Dissociator with Heaters. Although the dissociation process was optimized to get the highest possible number of viable cells, the resulting cell suspension still contained a significant amount of cell debris (fig. 1A) and red blood cells (fig. 1B), which hampers subsequent cell isolation and cultivation.

To overcome this drawback, we included a novel protocol for removal of debris and erythrocytes, which led to a substantial increase in the percentage of neural cells (fig. 1 C, D).
The optimized tissue dissociation process including removal of debris and erythrocytes yielded 2–4×10$^6$ living cells per adult mouse brain. The required reagents are now available as Adult Brain Dissociation Kit, mouse and rat.

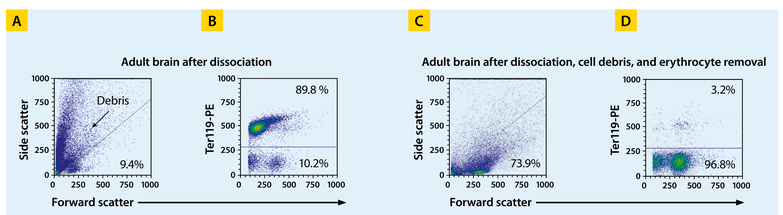


Figure 1

### 2 Isolation and cultivation of astrocytes from adult mouse brain tissue

After tissue dissociation using the Adult Brain Dissociation Kit, astrocytes were labeled with MACS MicroBeads coupled to antibodies specific for the astrocyte marker ACSA-2 (astrocyte cell surface antigen-2) and isolated using MACS Technology (fig. 2A). Cells were stained with Anti-ACSA-2-APC before and after separation (fig. 3B, C) for flow cytometry (MACSQuant® Analyzer) (fig. 2B). Enriched astrocytes showed a purity of 94±5% and a viability of 69±10.3%. A total number of 4×10$^5$±7.4×10$^4$ living astrocytes was obtained per adult mouse brain (n=15). Isolated astrocytes were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 on PLL/Laminin–coated 24-well glass bottom imaging plates. After 7 days, cells were fixed and subjected to immunocytochemical analysis using GLAST (red)– and GFAP (green)–specific antibodies. Cultivated cells formed a dense layer of GLAST/GFAP–positive astrocytes (fig. 2C), whereas only a very low number of neurons, oligodendrocytes, or microglia was detected (fig. 2D).

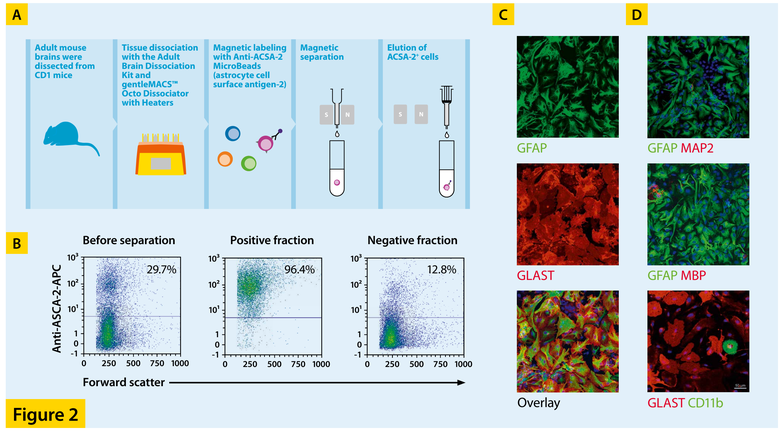


Figure 2

### 3 Isolation and cultivation of oligodendrocytes from adult mouse brain tissue

Oligodendrocytes were magnetically separated using the oligodendrocyte-specific Anti-O4 MicroBeads. Oligodendrocytes were enriched to a purity of 90±6.7% and a viability of 76.7±10.3% (fig. 3A). A total number of 1.2×10$^5$±2.5×10$^4$ living oligodendrocytes was obtained per adult mouse brain (n=7). Isolated cells were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 and 10 ng/mL FGF-2 and PDGF-AA on PLL-coated substrates. After 5 days, cells were fixed and stained using O4 (red)– and MOG (green)–specific antibodies (fig. 3B). Cultivated adult oligodendrocytes showed the typical branched morphology and almost no contaminating astrocytes, neurons, or microglia (fig. 3C).

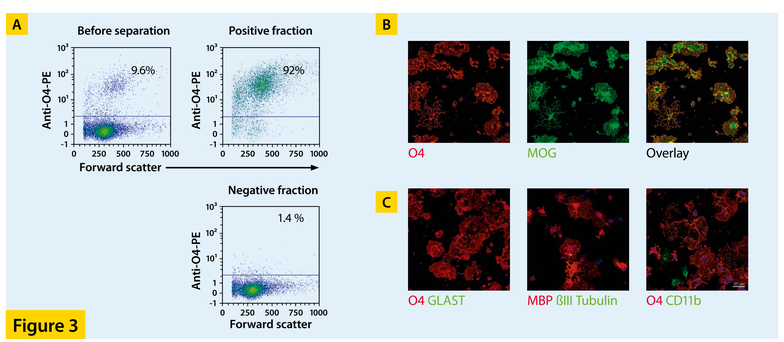


Figure 3

### 4 Isolation and cultivation of neurons from adult mouse brain tissue

Neurons were enriched by depletion of non-neuronal cells, using the Neuron Isolation Kit, mouse. Magnetically labeled non-neuronal cells were retained within an LS Column placed in a MACS Separator, while the highly enriched unlabeled neuronal cells were collected in the flow-through (fig. 4A). The original cell fraction and the isolated neuronal cells were stained with an antibody specific for the non-neuronal cell antigen and an astrocyte-specific antibody (fig. 4B). The neuronal cell fraction showed a high purity (92.2±1.1%) and a viability of 72±7.1%. The separation yielded 2×10$^5$±2.5×10$^4$ viable neuronal cells per whole mouse brain (n=3). Isolated neurons were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 on PLL-coated substrates and stimulated with 50 ng/mL BDNF on day 3 for 3–6 h. After 7 days cells were fixed and stained with antibodies against different neural cell types to determine purity of the neuronal cells. The culture showed a well grown neuronal network as indicated by MAP2 (green) and βIII Tubulin (red) staining (fig. 4C). Only very few MBP-positive oligodendrocytes, GLAST-positive astrocytes, or CD11b-positive microglia were detected (fig. 4D).

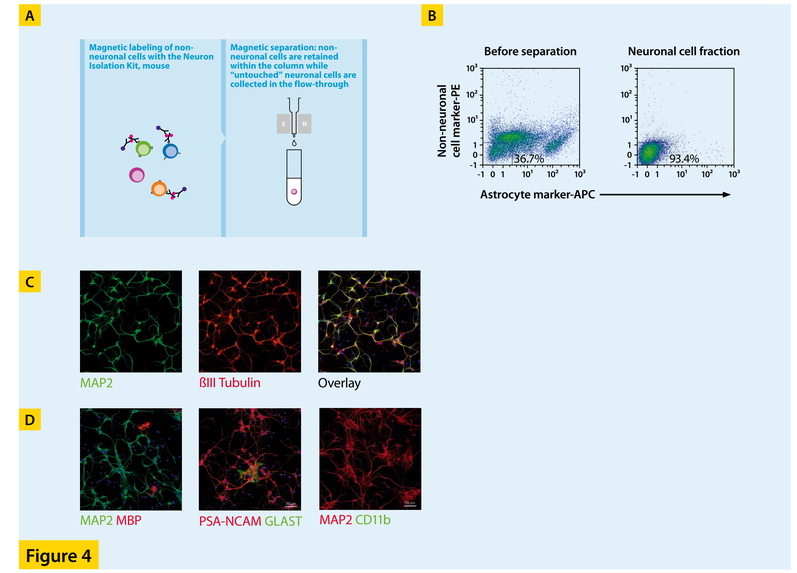


Figure 4

## Summary and outlook

- We present a novel technology that allows gentle automated dissociation of adult rodent brain tissue and leads to a high amount of viable single cells.
- The Adult Brain Dissociation Kit, mouse and rat enables for the first time the isolation of viable and functional adult astrocytes, oligodendrocytes, or neurons.
- The procedure including tissue dissociation and cell isolation takes only 4 hours to complete, avoiding tedious traditional cell culture–based separation, which takes up to 2 weeks.
- Highly purified adult astrocytes, oligodendrocytes, and neurons can be cultivated and applied to study the function of individual adult neural cells at the morphological and molecular level.