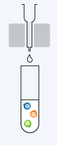
# Standardized tissue dissociation and removal of myelin debris optimize isolation of cells from human brain tumor

Sandy Reiß[1], Stephan Dützmann[2], Sandra Pennartz[1]
[1] Miltenyi Biotec GmbH, Friedrich-Ebert-Str. 68, 51429 Bergisch Gladbach, Germany | [2] Department of Neurosurgery, Johann Wolfgang Goethe University, Frankfurt am Main , Germany

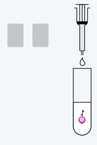
## Introduction

The quality of many experiments depends upon tissue dissociation as a first step. High yields of single cells with high viability are required, while antigen epitopes must be preserved for downstream applications involving antibody binding.
Reliable and standardized protocols are needed for research that focuses on the role of specific cell populations within a tumor and on the analysis of the carcinogenic potential with regard to the development of therapies.[1,2]
Here we present optimized methods for brain tumors, such as the incurable malignant glioblastoma multiforme, including tissue dissociation, sample preparation, and cell isolation.

**Magnetic labeling**
For **depletion of myelin debris,** the single-cell suspension is incubated with Myelin Removal Beads.
For **positive selection,** target cells are labeled with cell type-specific MicroBeads.



**Magnetic separation**
**Depletion:** Labeled myelin particles are retained in the column, while the flow-through contains the myelin-depleted fraction ready for subsequent applications.
**Positive selection:** Labeled target cells are retained in the column. The flow-through contains the negative fraction.



**Elution of the labeled cell fraction**
**Positive selection:** The column is removed from the magnetic field and labeled target cells are eluted.

MACS® Technology for cell separation. Either certain cells—or other suspension components—can be depleted, or cells can be positively selected according to certain surface molecule.

Figure 1

## Results

### 1 The Brain Tumor Dissociation Kit is ideal for the generation of single cells from human glioblastoma

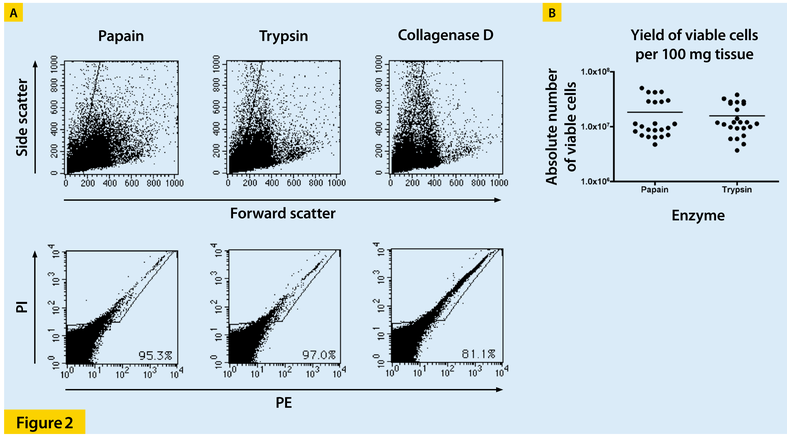



Figure 2

Human primary glioblastoma tissue was dissociated using the Brain Tumor Dissociation Kit (Miltenyi Biotec) based on papain or trypsin treatment, or a protocol based on collagenase D. Flow cytometric analysis shows the distribution of cells and myelin debris, as well as the percentage of dead cells: forward scatter (cell size), side scatter (granularity), and PI fluorescence (dead cells).
Use of collagenase D results in higher granularity of cells and lower viability compared to papain and trypsin. The yield of single cells after papain and trypsin treatment ranges from $5\times10^6$ to $5\times10^7$. Variability among experiments is in part due to the heterogeneity of the tumor samples.

### 2 The gentleMACS™ Dissociator facilitates tumor dissociation and increases reproducibility

In order to standardize and simplify the tissue dissociation procedure we developed a semi-automated protocol using the gentleMACS™ Dissociator.
Flow cytometric analysis shows that in combination with the Brain Tumor Dissociation Kit (P or T) both manual dissociation and the gentleMACS Dissociator produce high yields of single cells with excellent viability. However, tumor samples with tight texture require extensive pipetting and sometimes stick to pipette walls, whereas the gentleMACS Dissociator allows easy processing.

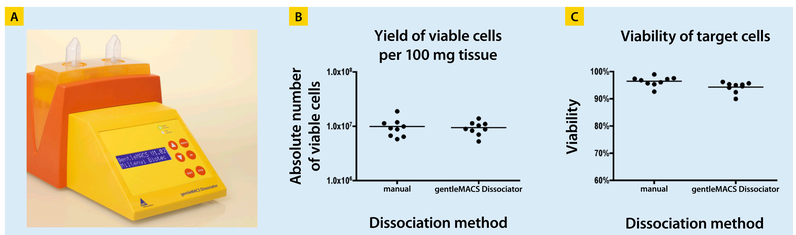



Figure 3

### 3 Removal of myelin debris and erythrocytes improves immunostaining

Human primary glioblastoma tissue was dissociated using the Brain Tumor Dissociation Kit. Flow cytometric analysis shows that single-cell suspensions of primary glioblastoma contain large amounts of myelin debris, other cellular debris, and erythrocytes. Because of the diversity of tumor samples the percentage of single, viable cells ranges from only 3% to 51%. Erythrocytes can amount to up to 92% of these viable cells.
Myelin Removal Beads (Miltenyi Biotec) were used in combination with LD Columns and QuadroMACS™ Separators to remove myelin debris (A). Anti-Glycophorin A-Biotin in combination with Anti-Biotin MicroBeads, LD Columns, and QuadroMACS Separators completely depleted erythrocytes. The MACSQuant® Analyzer was used for flow cytometric analysis. Dead cells were excluded from the analysis by PI fluorescence.
$1\times10^6$ cells from a single-cell suspension of human primary glioblastoma were fluorescently stained with CD44-APC and CD11b-PE.[3] Samples without or with prior removal of myelin debris and erythrocytes were compared.
Dot plots show that the removal of myelin debris and erythrocytes leads to more effective staining of CD44- and CD11b-positive cells (C, D). Dead cells were excluded by PI fluorescence.

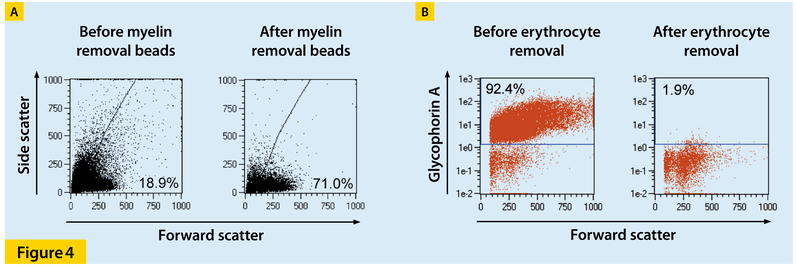



Figure 4

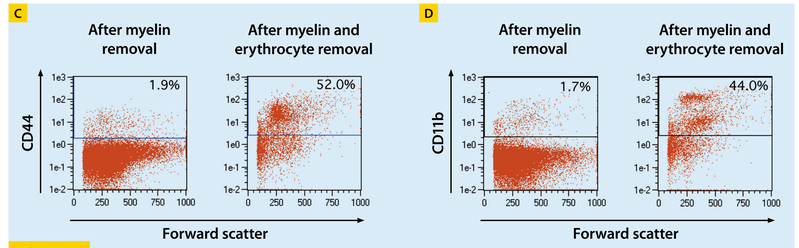



Figure 4

### 4 Isolation of microglia from optimally prepared samples leads to high purity and viability

Microglia were isolated from a single-cell suspension using MACS® Technology.[4] Dissociation of human primary glioblastoma was performed using the Brain Tumor Dissociation Kit (P) and the gentleMACS™ Dissociator. Myelin Removal Beads were used to deplete myelin debris. CD11b (Microglia) MicroBeads and an LS Column were used to achieve a purity of 97%. Purified microglia proliferated and showed normal morphology *in vitro*.

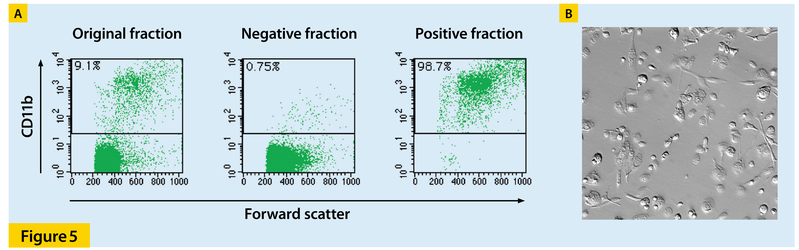



Figure 5

## Conclusion

- Brain Tumor Dissociation Kit and gentleMACS™ Dissociator allow the standardized generation of single-cell suspensions from human primary glioblastoma.
- Removal of myelin debris and erythrocytes from tumor samples is often indispensable for efficient antibody binding to target cells.
- Optimal preparation of human brain tumor samples results in successful immunomagnetic isolation of cells, e.g., microglia.

**References**

1. Son, M. J. *et al.* (2009) SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell 4: 440–52.
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