Semi-automated tissue dissociation and preserved epitope integrity optimize immunomagnetic sorting of neural cells

Sandy Reiß, Ina Herzig, Jürgen Schmitz, Andreas Bosio, Sandra Pennartz | Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

We have previously shown that using the Neural Tissue Dissociation Kit (Miltenyi Biotec) for enzymatic dissociation of whole mouse brain tissue or of specific regions, such as the subventricular zone, results in high yields of viable single cells. However, manual mechanical tissue dissociation causes fluctuations in the yield of viable cells due to pipetting at different speeds using fire-polished Pasteur pipettes with variable openings. In order to increase the reproducibility of enzymatic tissue dissociation and to facilitate sample processing, we performed the Neural Tissue Dissociation Kit protocol with a semi-automated mechanical dissociation system, named gentleMACS™ Dissociator (Miltenyi Biotec).

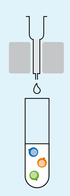
The loss of antigen epitopes can dramatically influence the performance of cell separations according to the expression of cell surface markers. It can either decrease the yield of target cells or the outcome might change when using a separation strategy combining several markers. Therefore, it is important to choose the appropriate protease for any experiment according to the antigen epitope which is used for isolation. Papain is often viewed as a mild protease, while trypsin treatment is regarded as harsh and causing detrimental effects on epitopes. We show that this perception does not apply to a number of antigen epitopes and that even the opposite can be the case.

gentleMACS™ Dissociator

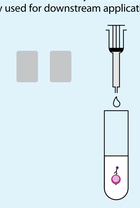
MACS® Procedure

Magnetic labeling
Cells of interest are labeled with MACS® MicroBeads in a short incubation step.

Magnetic separation
Labeled and unlabeled cells are separated on a MACS Column placed in the magnetic field of a MACS Separator. The flow-through can be collected as the non-magnetic, negative fraction.

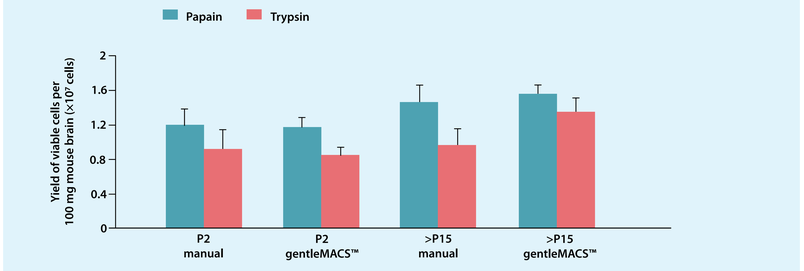


Elution of the labeled cell fraction
The separation column is removed from the magnetic field and the retained cells are flushed out as the magnetically labeled, positive fraction. Both fractions—labeled and unlabeled—can easily be isolated and directly used for downstream applications.



Results

1 Cell viability: gentleMACS™ Dissociator vs. manual dissociation

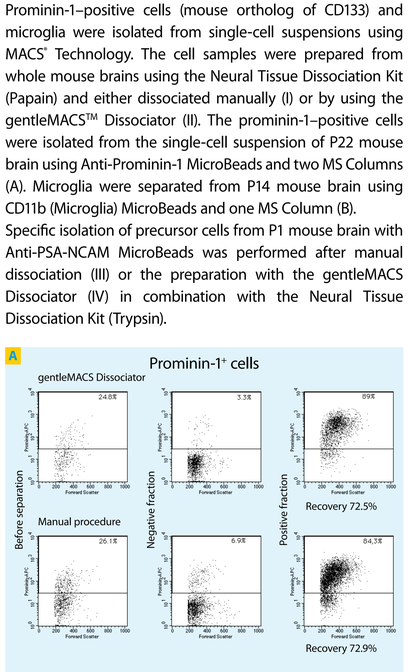


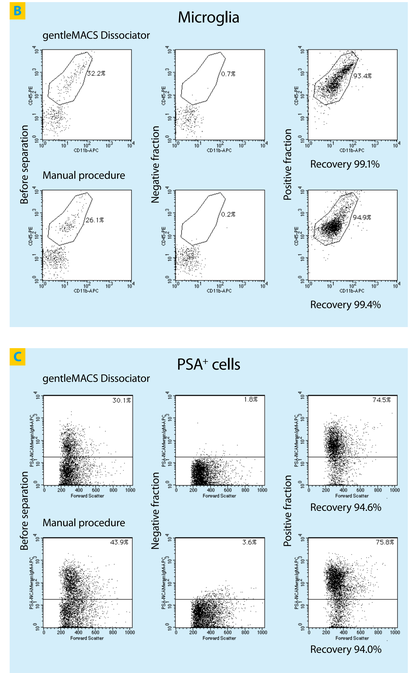
Whole brain tissue from P2 mice or mice older than P15 was dissociated using either the Neural Tissue Dissociation Kit (Papain) or the Neural Tissue Dissociation Kit (Trypsin), either manually or in combination with the gentleMACS™ Dissociator. The papain-based kit tended to yield higher numbers of viable cells than the trypsin-based kit.

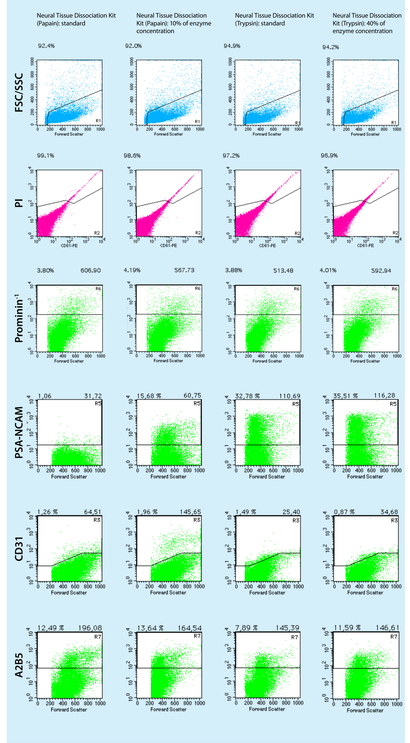
2 MACS® Cell Separations: gentleMACS™ Dissociator vs. manual dissociation

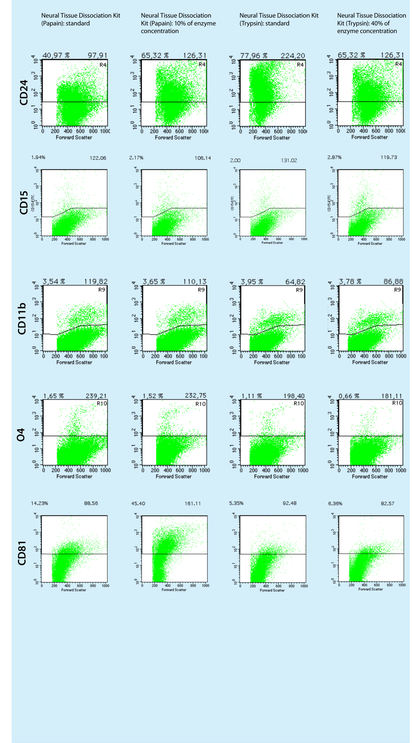
Prominin-1–positive cells (mouse ortholog of CD133) and microglia were isolated from single-cell suspensions using MACS® Technology. The cell samples were prepared from whole mouse brains using the Neural Tissue Dissociation Kit (Papain) and either dissociated manually (I) or by using the gentleMACS™ Dissociator (II). The prominin-1–positive cells were isolated from the single-cell suspension of P22 mouse brain using Anti-Prominin-1 MicroBeads and two MS Columns (A). Microglia were separated from P14 mouse brain using CD11b (Microglia) MicroBeads and one MS Column (B).

Specific isolation of precursor cells from P1 mouse brain with Anti-PSA-NCAM MicroBeads was performed after manual dissociation (III) or the preparation with the gentleMACS Dissociator (IV) in combination with the Neural Tissue Dissociation Kit (Trypsin).









3 Effects of different enzyme concentrations on epitope integrity

Flow cytometric analysis of several antibody stainings after dissociation of P1 brain tissue using the Neural Tissue Dissociation Kit (Papain) or the Neural Tissue Dissociation Kit (Trypsin) as well as both with reduced enzyme concentrations show differences in the percentage of labeled cells (on the left) and the mean fluorescence intensity (on the right). Thus, the type and the concentration of the protease used for dissociation play an important role regarding the integrity of antigen epitopes.

Antibody	Trypsin-sensitive?	Papain-sensitive?	Cell type
Prominin-1	Very weakly	No	Neural progenitor cells
PSA-NCAM	No	Yes	Neuronal or oligodendrocyte precursors
CD31	Yes	No	Endothelial cells
A2B5	Weakly	No	Glial precursors
CD24	No	Yes	Neuronal precursors, ependymal cells, erythrocytes
CD15 (LeX)	Very weakly	Very weakly	Neural progenitor cells
CD11b	No	No	Microglia
O4	Yes	Weakly	Immature oligoden-drocytes
CD81	Yes	Weakly	Microglia, endothelial cells, glia

Conclusion

- The gentleMACS™ Dissociator standardizes and facilitates mechanical tissue dissociation.
- Distinct antigen epitopes exhibit characteristic sensitivities to either trypsin or papain or both.
- The choice of enzyme for tissue dissociation depends primarily on the epitope of interest.
- The Neural Tissue Dissociation Kit (Papain) or the Neural Tissue Dissociation Kit (Trypsin) in combination with the gentleMACS Dissociator are optimal for the generation of single-cell suspensions from neural tissues, e.g. for subsequent cell isolation using MACS® Technology.

Supported by: Network of Excellence NeuroNe