Removal of myelin debris after tissue dissociation optimizes immunomagnetic sorting of cells from postnatal brain

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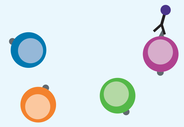
Introduction

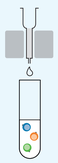
Myelin, a specialized membrane, ensheathes and insulates axons in the peripheral and central nervous system. In mice and rats, myelination begins around birth in the spinal cord and is completed in the brain during the first postnatal month[1]. In humans, myelin formation starts during the second half of fetal life in the spinal cord, peaks during the first year postnatally and can continue until 20 years of age[2].

When myelin containing neural tissue is dissociated, large quantities of myelin debris are generated. Myelin debris in single-cell suspensions was reported to considerably impair cell isolation and antibody staining[3,4]. So far, sucrose[5,6] has been used for the elimination of myelin. Here, we present a solution for myelin depletion based on magnetic cell sorting, MACS® Technology (see illustration below).



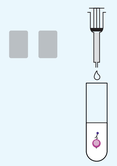
Magnetic labeling

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.



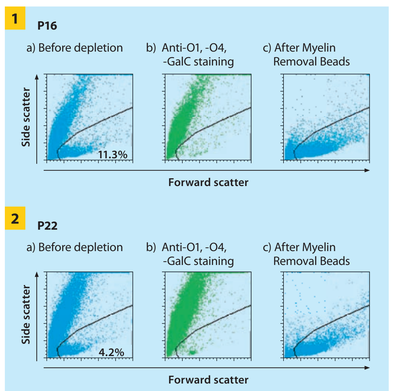
Elution of the labeled cell fraction

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 components—can be depleted, or cells can be positively selected for presence of a certain surface molecule.

Results

1 Myelin debris is abundant in single-cell suspensions

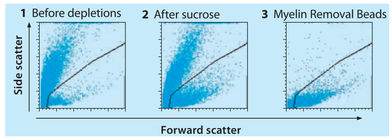


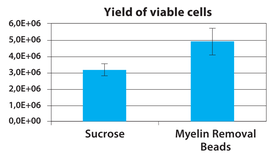
Mouse brains were dissociated using the Neural Tissue Dissociation Kit-P (NTDK-P, Miltenyi Biotec). Flow cytometrical analysis of the resulting single-cell suspensions shows the distribution of cells and myelin debris (a): forward scatter (cell size) and side scatter (granularity).

Single-cell suspensions from postnatal day (P) P16 (1) and P22 (2) mouse brains consist of large amounts of myelin membrane fragments, other cellular debris and only 11% and 4% cells, respectively. Particles outside the selected regions are positive for anti-O1, anti-O4 and anti-GalC antibodies that recognize glycolipids specifically contained in myelin membranes (b).

Myelin Removal Beads used with LD Columns and VarioMACS™ Separators efficiently remove myelin debris (c).

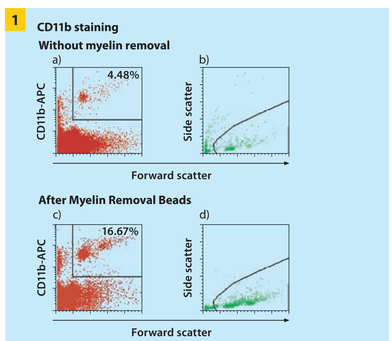
2 Myelin Removal Beads are more efficient than sucrose solution for myelin debris removal



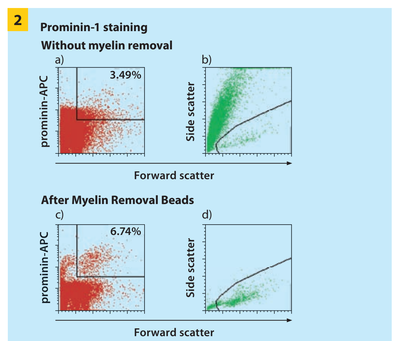


Myelin Removal Beads (Miltenyi Biotec) deplete myelin debris more thoroughly than sucrose solution. Dot plots show the amount of myelin debris and cells in forward and side scatter before depletion (1), after treatment with 10 mL of 0.9 M sucrose and centrifugation at 850×g for 10 minutes[6] (2) and after depletion using Myelin Removal Beads (3). Dead cells were labeled with PI and are not shown in the dot plots (n= 5).
The diagram illustrates that Myelin Removal Beads produce a higher recovery of viable cells from a starting population of 1×10^7 cells than treatment with sucrose solution.

3 Removal of myelin debris improves antibody stainings

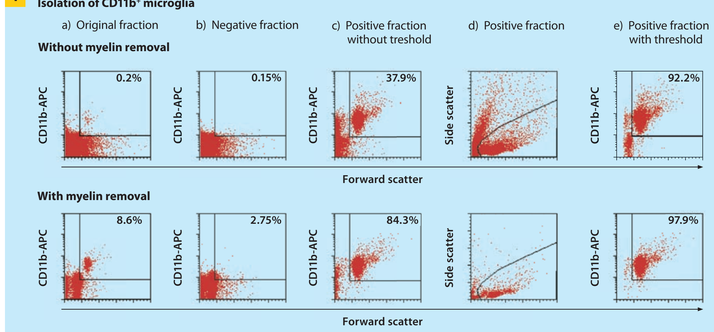


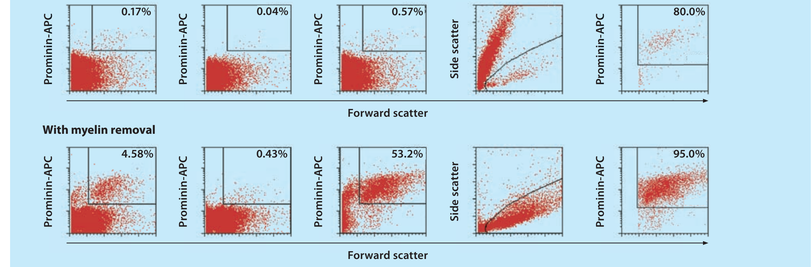
1×10^6 cells from a single-cell suspension of P22 mouse brain were stained with CD11b-APC (1) or anti-Prominin-1-APC (2) without and with previous myelin removal using Myelin Removal Beads. Dot plots 1a+c and 2a+c show that in samples with previous myelin removal, higher percentages of CD11b- and

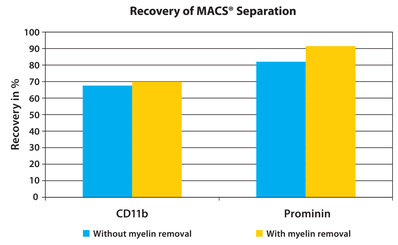


prominin-1-positive cells are stained, respectively. Dead cells were excluded using PI. Only the positive cells along with positive debris are displayed in dot plots 1b+d and 2b+d in side and forward scatters.

4 Removal of myelin debris increases efficiency of MACS® Separations







For MACS Separations using CD11b-MicroBeads (1a–e) or Anti-Prominin-1 MicroBeads (2a–e), P18 or P22 mouse brain, respectively, was dissociated using the NTDK-T. 1×10^7 cells from a single-cell suspension were directly used for separation or were submitted to myelin depletion using Myelin Removal Beads.
Comparing the separations from samples without and with myelin removal demonstrates that the recovery is higher for samples with previous myelin removal.
These data demonstrate a higher efficiency of cell separations in the absence of myelin debris.

Conclusions

- Myelin Removal Beads efficiently deplete myelin debris
- The recovery of viable cells after Myelin Removal Beads is higher than after sucrose solution
- Removal of myelin significantly improves the results of immunostainings and cell isolation experiments

References

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