MACS cell Separation : New technology on PUMA

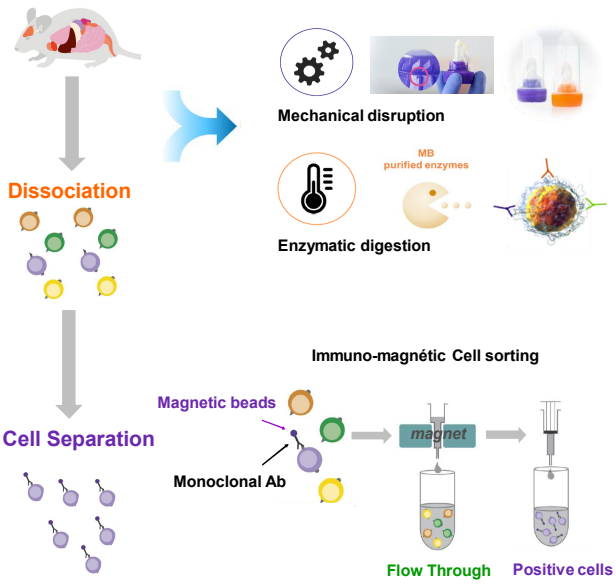
Marlène Maitre[1,2] – Hélène Doat[1,2]

[1]Inserm U1215, Neurocentre Magendie, Bordeaux-F33077, France
[2]Université de Bordeaux-F33077, France

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 worked a automated method for gentle dissociation of adult rodent brain tissue by combining mechanical dissociation using the gentleMACS™ with an optimized enzymatic treatment. Dissociation is followed by a procedure for removal of debris and erythrocytes, which is crucial for effective cell isolation. The standardised process allows rapid and reproducible dissociation of adult rodent brain tissue.
Protocols for MACS (MAgnetic Cell Sorter) isolation of astrocytes, oligodendrocytes and neurons at high purity have also been established.
So, we have developed a standardised process that allows for gentle and automated dissociation of adult rodent brain tissue and magnetic isolation with highly viable astrocytes, oligodendrocytes and neurons,

PRINCIPLE AND WORKFLOW

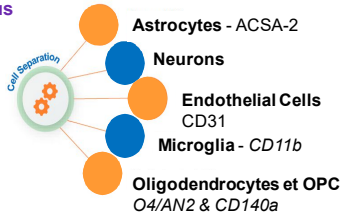


MATERIAL

Dissociation: GentleMACS™

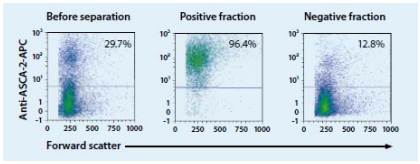
- Fast & reproductible
- Gentle epitope preservation for downstream target cell isolation or analysis
- Viable single cell suspensions
- Semi-automated cell separation

Cell Separation: MultiMACS Cell24 Plus



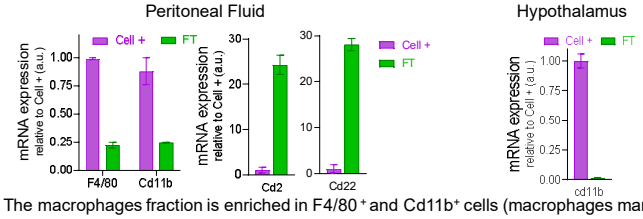
APPROVED PROTOCOLS

- **High purity separation of astrocytes in hippocampus by FACS analysis**



Live cells 88%
Astrocytes 96,4%

- **High purity separation of microglia by qPCR analysis**



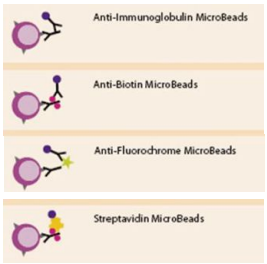
The macrophages fraction is enriched in F4/80⁺ and Cd11b⁺ cells (macrophages markers) and poor in Cd2⁺ and Cd22⁺ cells (lymphocytes markers).

RANGE OF POSSIBILITIES

Different selection processes with a wide range of possibilities

➢ Indirect positive isolation:

- **Useful in cases with dim surface marker expression**
- **Allows for amplification of signal**
- **Secondary antibody recognizes:**
  - **Fc portion of primary antibody**
  - **Biotin**
  - **FITC/PE/APC**
- **Streptavidin microbeads instead of secondary antibody (recognizes biotin on primary antibody)**

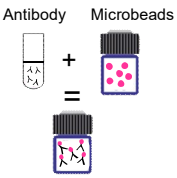


➢ Depletion :

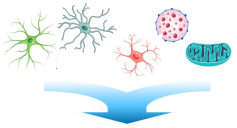
- **Unwanted cells are magnetically labelled and depleted**
- **Useful in following cases**
  - **Removal of unwanted cells**
  - **No specific antibody is available for target cell**
  - **Binding of antibody to target cell results in activations**

➢ Design your targets : MACSflex™ MicroBeads

- **Coupling microbeads of a variety of biomolecules: antibodies, proteins, oligonucleotides, peptides**
- **The fast coupling procedure allows you to use the coupled MicroBeads in downstream applications, such as epitope-tagged protein isolations and organelle isolations.**



DOWNSTREAM APPLICATIONS



Respirometry
Metabolics Analysis
Omics Analysis (PCR, NGS, Western blot...)
Cell Culture
Single-Cell Analysis (sorter, C1)
FACS

CONTACT

marlene.maitre@inserm.fr

SUMMARY AND OUTLOOK

PUMA propose a fast technology that allows automated and gentle dissociation of adult rodent brain tissue and allows to obtain a quantity of viable single cells with yields hight purity.
We are able to isolate viable and functional adult astrocytes, oligodendrocytes or neurons from adult brain mouse and rat with the principle Immuno-magnétic Cell sorting.
The standardized procedure, which includes tissue dissociation and cell isolation, takes only 4 hours. Note, the antigen must be extracellular and sufficiently represented.
Highly purified astrocytes, oligodendrocytes and adult neurons, can be cultured and used to study the function of individual adult neural cells to molecular level.
We can also propose: Mitochondria purification , Brain organoid dissociation, Lysosome isolation

PARTNERS

Nov-2022