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Enrichment and isolation of neurons from adult mouse brain for *ex vivo* analysis

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Highlights

- Enrichment of adult mouse neurons by FACS sorting
- Isolation of adult mouse neurons by magnetic-beads depletion of non-neuronal cells
- *Ex vivo* RNA analysis of adult mouse neurons

Abstract

Background: Isolation of neurons from the adult mouse CNS is important in order to study their gene expression during development or the course of different diseases.

New Methods: Here we present two different methods for the enrichment or isolation of neurons from adult mouse CNS. These methods are either based on flow cytometry sorting of eYFP expressing neurons, or by depletion of non-neuronal cells by sorting with magnetic-beads.

Results: Enrichment by FACS sorting of eYFP positive neurons results in a population of 62.4% NeuN positive living neurons. qPCR data shows a 3-5fold upregulation of neuronal markers. The isolation of neurons based on depletion of non-neuronal cells using the *Miltenyi* Neuron Isolation Kit, reaches a purity of up to 86.5%. qPCR data of these isolated neurons shows an increase in neuronal markers and an absence of glial markers, proving pure neuronal RNA isolation.

Comparison with Existing Methods: Former data related to neuronal gene expression are mainly based on histology, which does not allow for high-throughput transcriptome analysis to examine differential gene expression.

Conclusion: These protocols can be used to study cell type specific gene expression of neurons to unravel their function in the process of damage to the CNS.

Keywords: Neurons; Adult Mice; Isolation; RNA Isolation; Enrichment; FACS sorting; Magnetic-beads; *ex vivo*

1. Introduction

The central nervous system (CNS), including the brain and spinal cord, is one of the most complex tissues in the body, and consists of various cell types. Neuronal axons are wrapped with myelin sheaths and form close contacts with several other cells such as astrocytes, oligodendrocytes or microglia.