RNA extraction from sorted neuronal subtypes

PROTOCOL FOR:

Trehalose-enhanced isolation of neuronal sub-types from adult mouse brain

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See full protocol online: <https://benchtalk.biotechniques.com/users/8521-biotechniques-editors/posts/11115-protocol-for-rna-extraction-from-sorted-neuronal-subtypes>

Protocol Summary: Efficient isolation of specific, intact, living neurons from the adult brain is problematic due to the complex nature of the extracellular matrix consolidating the neuronal network. Here, we present significant improvements to the protocol for isolation of pure populations of neurons from mature postnatal mouse brain using fluorescence activated cell sorting (FACS). The 10-fold increase in cell yield enables cell-specific transcriptome analysis by protocols such as nanoCAGE and RNA seq.

Protocol Schematic: Improved neuron and RNA acquisition using our optimized protocol. Images taken before sorting (A,B) for standard protocol (A) and optimized protocol with Trehalose (B). Note the dead cells in un-dissociated tissue (arrow) as well as dead single cells without projections (arrowhead) in the standard protocol. Dissociated intact live neurons (arrows and inset) as well as dead dissociated neurons (arrowhead) are seen with the optimized protocol. (C–F) Images taken after sorting for optimized protocol. Note the intact neuron (C,D) and cells with small (arrow) or no projections (arrowhead) (E,F) in the after sort images from optimized protocol. Scale bar: 20 μ m. Bioanalyzer profiles of RNA extracted from sorted pyramidal neurons isolated using the standard protocol (G) and optimized protocol with Trehalose (H). Note the size of

the 28S peak (arrow) is smaller than the 18S peak (arrowhead) in the RNA extracted using the standard method, suggesting RNA degradation with the standard protocol. (I) Chart depicting the yields of fluorescent pyramidal cells using the standard method (n = 2) and our optimized method (n = 6), (Cell numbers shown in Tables 1 and 2). Cell numbers are increased 10-fold when trehalose is used. ** P < 0.001; Student's t-test, error bars represent s.d.