Mar 15, 2024

Isolation of NeuN+ cells from brain tissue (for CUT and RUN)

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l27pejg1y/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.4r3l27pejg1y/v1

Protocol Citation: anita.adami 2024. Isolation of NeuN+ cells from brain tissue (for CUT and RUN). **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l27pejg1y/v1

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Protocol status: Working
We use this protocol and it's working

Created: March 03, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 78067

Keywords: ASAPCRN, brain tissue, preparation for cut, cell, tissue

Funders Acknowledgements:
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-000520
Swedish Research Council
Grant ID: 2018-02694
Swedish Brain Foundation
Grant ID: FO2019-0098
Cancerfonden
Grant ID: 190326
Barncancerfonden
Grant ID: PR2017-0053
NIHR Cambridge Biomedical Research Centre
Grant ID: NIHR203312
Swedish Society for Medical Research
Grant ID: S19-0100
National Institutes of Health
Grant ID: HG002385
Swedish Research Council
Grant ID: 2021-03494
Swedish Research Council
Grant ID: 2020-01660

Abstract

This protocol describes the steps to isolate NeuN+ cells from brain tissue in preparation for CUT and RUN

Isolation of NeuN+ cells

45m

1 Nuclei were isolated from frozen tissue as described **here**.

2 Before FACSing, nuclei were incubated with Recombinant Alexa Fluor® 488 Anti-NeuN antibody [EPR12763] - Neuronal Marker (Abcam, Cat# ab190195, RRID:AB_2716282) at a concentration of 1:500 for 00:30:00 On ice as according to **Spalding, K.L. et al. (2013).** 30m

3 The nuclei were run through the FACS at 4 °C with a low flow rate using a 100 mm nozzle and 300.000 nuclei Alexa Fluor – 488 positive nuclei were sorted.

4 The sorted nuclei were pelleted at 1,300 x g for 00:15:00 and resuspended in 1 mL of ice-cold nuclear wash buffer (20 mM HEPES, 150 mM NaCl, 0.5 mM spermidine, 1x cOmplete protease inhibitors, 0.1% BSA) and 10 µL per antibody treatment of ConA-coated magnetic beads (Epicypher) added with gentle vortexing (Pipette tips for transferring nuclei were pre-coated with 1% BSA), to perform **CUT&RUN**. 15m