Jan 07, 2020

Nuclei Isolation for SNARE-seq2

DOI
dx.doi.org/10.17504/protocols.io.8tvhwn6

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8tvhwn6

Protocol Citation: Carter Palmer, Christine Liu, Jerold Chun 2020. Nuclei Isolation for SNARE-seq2 . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.8tvhwn6**

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

Created: October 28, 2019

Last Modified: January 07, 2020

Protocol Integer ID: 29269

Keywords: Brain, Nuclei, Isolation, Single-Cell

Abstract

This protocol is intended to be used for the isolation of nuclei from fresh-frozen brain tissue in preparation for analysis by Single-Nucleus Chromatin Accessibility and mRNA Expression sequencing (SNARE-seq). It has been applied to tissues from mouse, marmoset, and human.

Guidelines

This protocol is designed specifically for isolating tissue for SNARE-seq. RNA stability is considered at every step. Samples are kept on ice throughout the process, all centrifuges are pre-chilled to 4 ^{O}C before use, and RNase Away is used to spray down all surfaces and pipets before use.

Materials

MATERIALS

- Sucrose **Fisher Scientific** **Catalog #**S3-212
- Protease Inhibitor Tablets cOmplete Mini EDTA free **Roche** **Catalog #**11836170001
- RNase Inhibitor **Takara Bio Inc.** **Catalog #**2313A
- Bovine Serum Albumin **Gemini Bio-Products** **Catalog #**700-107P
- Magnesium acetate tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M5661
- Calcium chloride dihydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C5080
- Ethylenediaminetetraacetic acid (EDTA) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**EDS
- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8787
- 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific** **Catalog #**28906
- DAPI **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**10236276001
- 50um filters **Sysmex** **Catalog #**04-0042-2317
- Tissue Homogenizer **Catalog #**358005