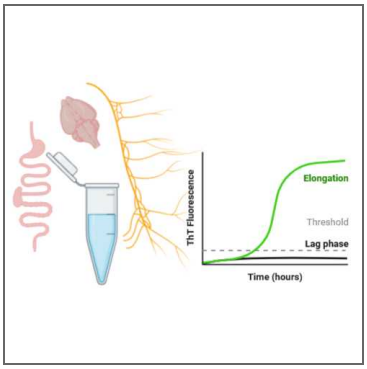

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Preparation of mouse tissue homogenates for RT-QuIC assay

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ABSTRACT

This protocol is designed for a standardized and efficient procedure to homogenize mouse tissue, conducive for RT-QuIC analysis. The process involves treating samples with PBS mixed with Triton-X100, followed by homogenization using a prob-tip sonicator. Centrifugation is then employed to remove detergent-insoluble fraction, optimizing the tissue for subsequent
analysis. The use of Triton-X100, a mild detergent, ensures the release of alpha-synuclein aggregates, enhancing signal specificity without compromising structural integrity. The protocol's significance lies in addressing the critical need for a reliable method in preparing mouse tissue for RT-QuIC analysis, with the distinct signal generated in assays validating its efficacy.

PROTOCOL MATERIALS

Low Protein Binding Collection Tubes (1.5 mL) **Thermo Fisher Catalog #90411**

Step 1

Roche PhosSTOP EasyPack **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4906837001**

Step 4

Roche cOmplete Tablets EDTA-free, EasyPack **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4693132001**

Step 4

Protein LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108116**

In 2 steps

BEFORE START INSTRUCTIONS

The tissues need to be perfused with fresh PBS and flash-frozen in liquid nitrogen

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

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Last Modified: Jan 15, 2024

PROTOCOL integer ID:
91538

Keywords: ASAPCRN, alpha-synuclein, tissue homogenation, rt-quic

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1 Measure the weight of each eppendorf tube using analytical scale and label the tube with assigned number

Protein LoBind Tubes, 1.5 mL **VWR International Catalog #0030108116**

Low Protein Binding Collection Tubes (1.5 mL) **VWR International Catalog #90411**

2 Cut a tissue slice (around 100-200 mg) with a disposable blade on a plastic plate lid wrapped in a foil (all on ice), transfer to the Eppendorf protein low-binding tube (with known weight)

Safety information

biohazard; place disposable blades into biohazard needle container; material should be thrown away into biohazard bags previously placed into tube or a 15 mL falcon (secondary container)

3 Measure the weight of the tissue piece in a tube using analytical scales
Subtract weight of the tube to obtain the final weight of the brain slice (indicate the weight in the table)

4 Add 20 vol weight/volume of PBS buffer containing 1%Tx100 and protease/phosphatase inhibitors (

Roche PhosSTOP EasyPack **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4906837001**

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Roche cOmplete Tablets EDTA-free, EasyPack **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4693132001**
(for example, 100 mg – 5 mL) - transfer to 15 mL falcon tube;

5 Sonicate tissue samples for 00:01:00, 5 sec ON and 15 sec OFF (On ice); 1m
Use 0.16 inch microtip for homogenization

Equipment

Fisherbrand Model 120 Sonic Dismembrator	NAME
Sonicator	TYPE
Fisherbrand	BRAND
FB120110	SKU
https://www.fishersci.com/shop/products/fisher-scientific-model-120-sonic-dismembrator-4/p-3974654	LINK
Equipped with 1/8" probe	SPECIFICATIONS

6 Spin down sonicated samples at 10.000 x g , 4 °C , 00:10:00 10m

7 Transfer supernatant into a new 15 mL falcon tube;

8 Aliquot the samples in lobind eppendorf
Protein LoBind Tubes, 1.5 mL **VWR International Catalog #0030108116** tubes and store at
-70 °C