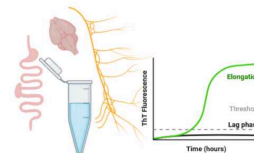


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# 🌐 Preparation of mouse tissue homogenates for RT-QulC assay

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**Protocol status:** Working

**We use this protocol and it's working**

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**Last Modified:** May 31, 2024

**Protocol Integer ID:** 91538

**Keywords:** ASAPCRN, alpha-synuclein, tissue homogenation, rt-quick, preparation of mouse tissue homogenate, preparing mouse tissue, mouse tissue for rt, synuclein aggregate, mouse tissue homogenate, mouse tissue, quick analysis, quick, tissue homogenate, treating sample, 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

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

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

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

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

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

## Troubleshooting

### Before start

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



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

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


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

 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 );  
Use 0.16 inch microtip for homogenization

1m



## 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 **Eppendorf Catalog #0030108116** tubes and store at

 -70 °C