Mar 03, 2025

Extraction of alpha-synuclein filaments from brain

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlk9z25v5r/v1

Yang Yang[1]

[1]Van Andel Research Institute

Yang Yang
Van Andel Research Institute

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlk9z25v5r/v1

Protocol Citation: Yang Yang 2025. Extraction of alpha-synuclein filaments from brain. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk9z25v5r/v1

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

Created: February 28, 2025

Last Modified: March 03, 2025

Protocol Integer ID: 123601

Keywords: synuclein filaments from brain, synuclein filament, synuclein filaments from postmortem, synuclein aggregates from other cellular component, synuclein assembly, synuclein aggregate, hallmark of neurodegenerative disorder, synuclein, biochemical characterization of these filament, neurodegenerative disorder, filament, filament morphology, minimal disruption to filament morphology, including tissue homogenization, biochemical characterization, parkinson, using cryo, extraction of alpha, tissue homogenization, biochemical assay, potential therapeutic targeting, dementia with lewy body

Disclaimer

No

Abstract

The accumulation of α-synuclein filaments is a hallmark of neurodegenerative disorders such as Parkinson's disease and dementia with Lewy bodies. Structural and biochemical characterization of these filaments is crucial for understanding their role in disease progression. Here, we present a detailed protocol for the extraction of α-synuclein filaments from postmortem human and animal brain tissue, enabling downstream structural analysis using cryo-electron microscopy (cryo-EM) and biochemical assays. This method involves a series of steps, including tissue homogenization, differential centrifugation, and detergent-based fractionation to selectively enrich for filamentous α-synuclein. The protocol ensures minimal disruption to filament morphology while effectively separating α-synuclein aggregates from other cellular components. This approach facilitates the study of strain-specific conformations, interactions with co-factors, and potential therapeutic targeting of pathogenic α-synuclein assemblies.

Extraction of alpha-synuclein filaments from brain

1 Homogenize and release the filaments from the tissue.

1.1 Cut and weight the tissue (0.2g-0.5g).

1.2 Tissues were homogenized in 20 vol(v/w) extraction buffer of 10 mM Tris-HCl, pH 7.5, 0.8 M NaCl, 10-20% sucrose and 1 mM EGTA.

1.3 Homogenates were brought to 2% sarkosyl and incubated for 60 min at 37 °C.

1.4 Following 10 min centrifugation at 10,000g, the supernatants were spun at 100,000g (45,000rpm) for 40-60 min.

2 Further enrich for filamentous α-synuclein and remove other components.

2.1 The pellets were resuspended in 500 µl/g extraction buffer and centrifuged at 3,000g for 5 min.

2.2 The supernatants were diluted threefold in buffer 2: 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl, 10% sucrose and 0.2% sarkosyl, and spun at 45,000 rpm for 30 min.

2.3 Resuspend the pellet with buffer 3 (100 ul/g tissue). Current concentration is for Cryo-EM, 1:10 dilution for Western Blot or negative staining.

2.4 For further analysis, the samples were centrifuged at 3,000 g for 2 min.

Protocol references

Yang Y, Shi Y, Schweighauser M, Zhang X, Kotecha A, Murzin AG et al. (2022) Structures of a-synuclein filaments from human brains with Lewy pathology. Nature 610: 791-795.