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Alpha-synuclein Seed Amplification Assay

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Sunil Srivastav[1], Davin Henderson[2], Jessika Suescun Ocampo[1], Divya Onkar Mondhe[1], * Richard Gordon[1]

[1]School of Biomedical Science, Translational Research Institute, Queensland University of Technology, Brisbane, 4102, Australia;
[2]CWD Evolution LLC, Fort Collins, CO, United States

Sunil Srivastav
Queensland University of Technology

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Protocol status: Working
We have been using this protocol successfully, and it is working well.

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Abstract

Preparation of fecal samples for alpha-synuclein seed amplification assay.

Materials

1. RT-QuIC buffer
2. alpha-synuclein substrate
3. optic bottom 96 well plates
4. Fluorometer

Protocol

1 Homogenize fecal samples in phosphate buffered saline

2 Centrifuge the homogenate and carefully remove the supernatant.

3 To the supernatant add metal-ion nanoparticles and resuspend them.

4 Next, perform magnetic separation of α-synuclein seeds.

5 Add these seeds to the 96 well plate and now resuspend them with Real-time quaking-induced conversion (RT-QuIC) buffer containing α-synuclein substrate.

6 Using a standard fluorometer, take fluorescence readings with an excitation of 450 nm and emission of 480 nm.

7 After a few hours are elapsed, an increase in fluorescence intensity could be observed, indicating alpha-synuclein seed amplification.

Protocol references

1. L. Concha-Marambio, S. Pritzkow, M.Shahnawaz, C. M. Farris, C. Soto, Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid. *Nat Protoc* **18**, 1179-1196 (2023).
2. D. M. Henderson *et al.*, Detection of chronic wasting disease prion seeding activity in deer and elk feces by real-time quaking-induced conversion. *J Gen Virol* **98**, 1953-1962 (2017).
3. N. D. Denkers, D. M. Henderson, C. K. Mathiason, E. A. Hoover, Enhanced prion detection in biological samples by magnetic particle extraction and real-time quaking-induced conversion. *J Gen Virol* **97**, 2023-2029 (2016).