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RT-QuIC alpha-synuclein

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DISCLAIMER

ABSTRACT

This protocol is for the detection of prionoid alpha-synuclein forms in human cerebrospinal fluid using the Real Time Quacking-Induced Conversion method (RT-QuIC). The protocol is adapted from Marco J. Russo, Christina D. Orru, Luis Concha-Marambio, Simone Giaisi et al., 2021 (doi:10.1186/s40478-021-01282-8) and Concha-Marambio et al., 2019 (doi:10.1007/978-1-4939-9124-2_4).
This assay is for research use only and not diagnostics.

EXTERNAL LINK

https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-021-01282-8,
https://link.springer.com/protocol/10.1007/978-1-4939-9124-2_4

PROTOCOL CITATION

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https://protocols.io/view/rt-quic-alpha-synuclein-cfs3tngn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Russo MJ, Orru CD, Concha-Marambio L, Giaisi S, Groveman BR, Farris CM, et al. High diagnostic performance of independent alpha-synuclein seed amplification assays for detection of early Parkinson's disease. Acta Neuropathol Commun. BioMed Central; 2021;9:1–13. Concha-Marambio L, Shahnawaz M, Soto C. Detection of Misfolded α-Synuclein Aggregates in Cerebrospinal Fluid by the Protein Misfolding Cyclic Amplification Platform. 2019. p. 35–44.

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MATERIALS TEXT

- BMG Technologies Omega FLUOstar Reader or similar fluorometer
- regular lab equipment (balance, pHmeter, pipetts, tips, tubes)
- COSTAR 96-well ELISA plates (Corning, cat# 3916)
- MicroAmp Film (Applied Biosystems, cat# 4311971)
- Si_3N_4 beads 2.38 mm
- molecular biology grade nuclease free water
- 0.5 M PIPES pH 6.5
- 5 M NaCl
- Thioflavin T dissolven in water
- recombinant C-terminal His-tag alpha synuclein
- 1% BSA
- 5 N NaOH and 1N HCl for pH adjustment

DISCLAIMER:

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Bead preparation

1 Beads are blocked with 1% BSA in 100mM PIPES for 1 hr and washed twice with PIPES.

Preparation of Reaction buffer

2 Reaction mixture is prepared calculating 200 µl per well with the following final concentrations:
- 0.3 mg/mL recombinant alpha-synuclein
- 0.5 M NaCl
- 100 mM PIPES buffer
- 5 µM ThT

Seeting up the assay

3 One bead is placed in each well of the assay plate. 160 µl reaction buffer and 40 µl CSF are carefully pipetted in each well. Samples are assessed in triplicates.

4 Plate is covered with the film and creases are removed manually.

5 Plate is placed in the plate reader and incubated for 240 hrs at 37 °C in cycles of 1 min shaking at 500rpm, 29 mins incubation and fluorescence measurements are taken after every incubation cycle at 440ex/490emm.

Data analysis

6 A sample is considered positive when it crosses a fluorescence threshold established at 3 standard deviations above baseline. 3/3 wells are positive and negative when 1/3 samples are positive while 2/3 is considered as incoclusive.

7 Relative fluorescence units measured at 490 are plotted versus the time in hours and should present a classical exponantial curve with lag phase and plateau. Kinetics parameters obtained from the curves include maximum fluorescence, time to reach 50% of maximal fluoresence and time to reach the established threshold.