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RT-QuIC alpha-synuclein

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We use this protocol and it's working

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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.

Materials

- 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

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.