NOV 15, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldmqkol5b/v1

Protocol Citation: mathew.horrocks 2023. SAVE imaging of protein aggregates in cerebrospinal fluid. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldmqkol5b/v1

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

Created: Nov 15, 2023

SAVE imaging of protein aggregates in cerebrospinal fluid

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ABSTRACT

This protocol describe the procedures used to clean a coverglass surface and perform ThT detection of protein aggregates in CSF (SAVE imaging) on a TIRF microscope.

MATERIALS

Equipment:

- Diener Electronic ZeptoOne Plasma Cleaner
- Oxford Nanoimager

Consumables:

- Coverslips (Merck, C9056-1CS)
- 0.02-micron syringe filters (Merck, WHA68092002)
- Frame-Seal slide chambers (9 × 9 mm^2, Biorad, Hercules, CA, SLF-0601).
- Eppendorf Protein LoBind tubes (Merck, EP0030108094)

Reagents:

- Phosphate buffered saline (Merck, P4417)
- Thioflavin T (Merck, 596200-500MG)
- Ethanol (Sigma-Aldrich, 459836)
- Poly-l-lysine (70 000–150 000 molecular weight, Sigma-Aldrich, P4707-50 ML)

Last Modified: Nov 15, 2023

PROTOCOL integer ID: 90959

Preparation of ThT

1 Prepare approx. 4 mM stock of ThT in 100% ethanol and vortex extensively (approx. 1 hour).

2 Prepare approx. 200 uM ThT dilution in PBS, vortex thoroughly (approx. 20 mins) and filter through 0.02-micron filter.

3 Measure concentration of ThT preparation using a DeNovix spectrophotometer (extinction coefficient 36,000 M^{-1} cm^{-1} at 412 nm).

4 Prepare a 50 uM working stock in 0.02-micron filtered PBS

Addition of sample for imaging

5 Add 5 uL CSF, 5 uL ThT working stock, and 40 uL PBS to an eppendorf tube.

6 Add 50 uL of the sample prepared in the previous step to the PLL-treated coverslips, and incubate for 1h.

7 Rinse with PBS.

8 Add 100 uL of 5 uM ThT to the coverslip.

Imaging

9 Acquire an 8 x 8 grid of 200-micron spaced fields of view per well by total internal reflection fluorescence microscopy using the ONI Nanoimager with 100x/1.4 oil immersion objective lens. Samples are excited at 405 nm, 50 frames captured per field in each channel at 20 frames s^{-1}.