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SAVE imaging of protein aggregates in cerebrospinal fluid

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Protocol status: Working

We use this protocol and it's working



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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 ($9 \times 9 \text{ mm}^2$, 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)



Preparation of ThT

- 1 Prepare approx. 4 mM stock of ThT in 100% ethanol and vortex extensively (approx. 1 hour).
- 2 Prepare approx. 200 μ M 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 $\text{M}^{-1} \text{cm}^{-1}$ at 412 nm).
- 4 Prepare a 50 μ M working stock in 0.02-micron filtered PBS

Addition of sample for imaging

- 5 Add 5 μ L CSF, 5 μ L ThT working stock, and 40 μ L PBS to an eppendorf tube.
- 6 Add 50 μ L of the sample prepared in the previous step to the PLL-treated coverslips, and incubate for 1h.
- 7 Rinse with PBS.
- 8 Add 100 μ L of 5 μ M ThT to the coverslip.

Imaging

- 9 Acquire an 8×8 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} .