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

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Amyloid beta (Aβ) aggregates N-terminal labeling

Patricia Yuste Checa¹, F Ulrich Hartl¹

¹Department of Cellular Biochemistry, Max Planck Institute of Biochemistry



Patricia Yuste-Checa

ABSTRACT

This protocol details how to efficiently label protein aggregates at the N-terminal using Amyloid beta as example.

ATTACHMENTS

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MATERIALS

Buffers:

- Labeling buffer: [M] 0.1 Molarity (M) sodium bicarbonate buffer pH 8.3
- 1x PBS pH 7.2
- Dimethyl sulfoxide (DMSO)
- pHrodo Red Succinimidylester Thermo Fisher Scientific Catalog #P36600



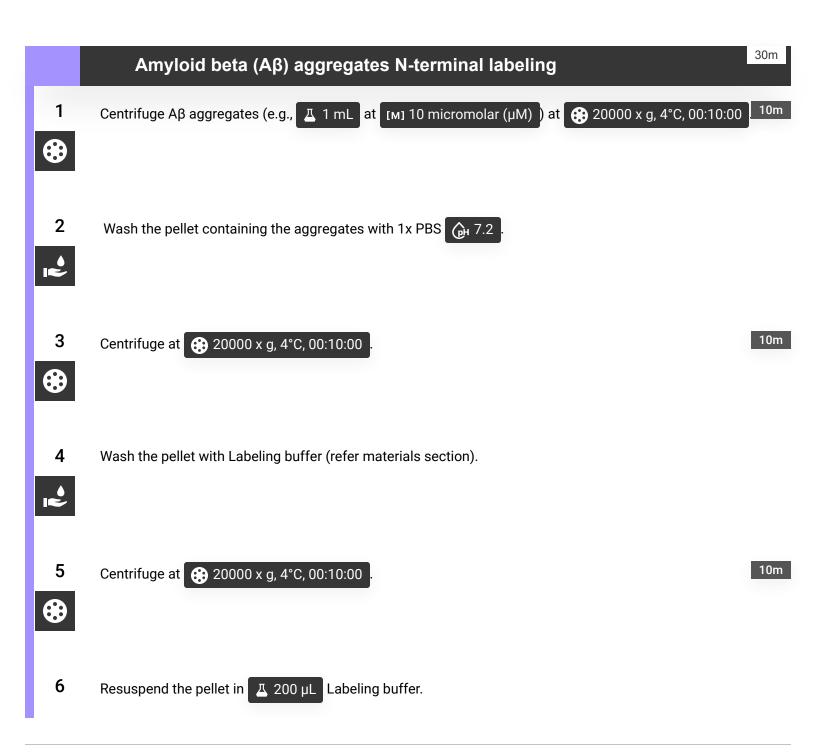
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- 7 Dissolve the dye (Alexa488 (A488) NHS ester, Thermo Fisher Scientific, A20000; pHrodo Red Succinimidylester, Thermo Fisher Scientific, P36600) in DMSO.
 - 7.1 With a pipette tip gently touch the dye powder which will stick to the tip.



- 7.2 Immerse the tip in some DMSO previously dispensed in a tube.
- 7.3 Repeat the procedure several times until the solution reaches the desired color.

Note

If labeling a protein for uptake assays analyzed by flow cytometry, it is recommended to use A488 because the A488 signal outside the cell can be easily quenched by adding Trypan blue right before measurement. pHrodo red dye is a pH sensitive dye which fluoresces brightly only in acidic environments and therefore can be used to specifically monitor phagocytosis and endocytosis.

8 Quantify diluted dye concentration by nanodrop. Dilute the sample in water to reach a λ <1 for an accurate measurement.

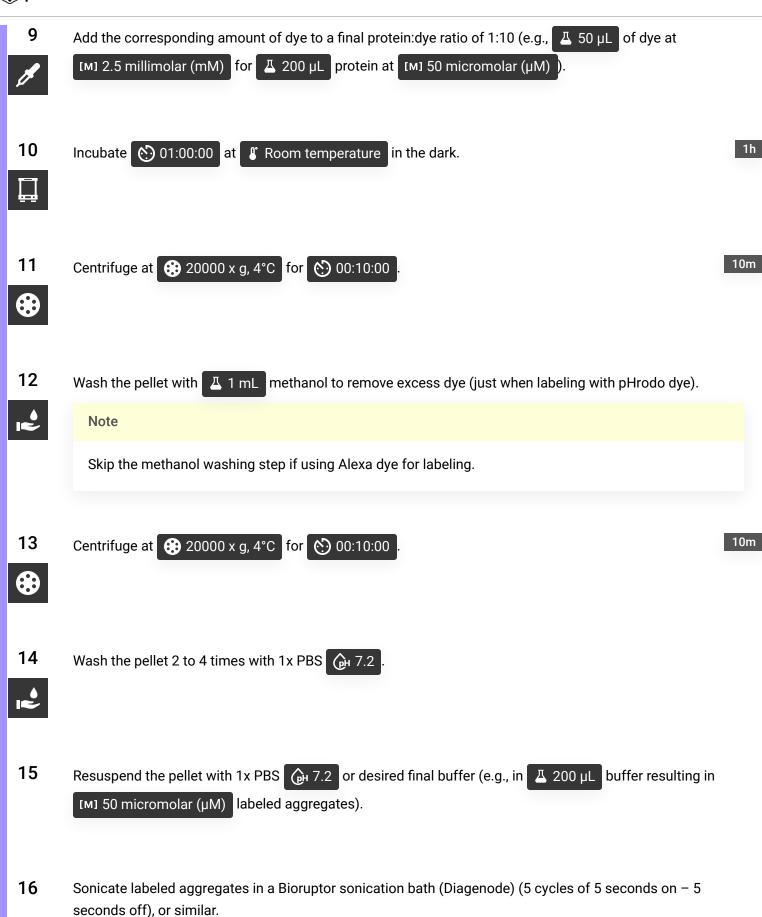
Note

Physical characteristics of the dyes to be set in the nanodrop:

Alexa488: Absorbance maximum (λ max): 495 nm; Extinction coefficient (ϵ): 71,000 cm⁻¹M⁻¹; Correction factor at 280 nm (CF₂₈₀): 0.11; Correction factor at 260 nm (CF₂₆₀): 0.3.

pHrodo Red: Absorbancemaximum (λmax): 560 nm; Extinction coefficient (ε): 65,000 cm⁻¹M⁻¹; Correction factor at 280 nm (CF₂₈₀): 0.12; Correction factor at 260 nm (CF₂₆₀): 0.36.

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Note

This protocol can be used for other aggregates like Tau or $\alpha\mbox{-Synuclein}$ aggregates.