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We use this protocol and it's working

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Amyloid beta (Aβ) aggregates N-terminal labeling

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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)

Alexa 488 NHS ester **Thermo Fisher Scientific Catalog #A20000**

pHrodo Red Succinimidylester **Thermo Fisher Scientific Catalog #P36600**

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Amyloid beta (Aβ) aggregates N-terminal labeling

30m

1 Centrifuge Aβ aggregates (e.g., 1 mL at [M] 10 micromolar (µM)) at 20000 x g, 4°C, 00:10:00. 10m

2 Wash the pellet containing the aggregates with 1x PBS pH 7.2.

3 Centrifuge at 20000 x g, 4°C, 00:10:00. 10m

4 Wash the pellet with Labeling buffer (refer materials section).

5 Centrifuge at 20000 x g, 4°C, 00:10:00. 10m

6 Resuspend the pellet in 200 µL Labeling buffer.

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 (λmax): 495 nm; Extinction coefficient (ε): 71,000 $cm^{-1}M^{-1}$; Correction factor at 280 nm (CF_{280}): 0.11; Correction factor at 260 nm (CF_{260}): 0.3.

pHrodo Red: Absorbancemaximum (λmax): 560 nm; Extinction coefficient (ε): 65,000 $cm^{-1}M^{-1}$; Correction factor at 280 nm (CF_{280}): 0.12; Correction factor at 260 nm (CF_{260}): 0.36.

9 Add the corresponding amount of dye to a final protein:dye ratio of 1:10 (e.g., 50 µL of dye at [M] 2.5 millimolar (mM) for 200 µL protein at [M] 50 micromolar (µM)).

10 Incubate 01:00:00 at Room temperature in the dark. 1h

11 Centrifuge at 20000 x g, 4°C for 00:10:00. 10m

12 Wash the pellet with 1 mL methanol to remove excess dye (just when labeling with pHrodo dye).

Note

Skip the methanol washing step if using Alexa dye for labeling.

13 Centrifuge at 20000 x g, 4°C for 00:10:00. 10m

14 Wash the pellet 2 to 4 times with 1x PBS pH 7.2.

15 Resuspend the pellet with 1x PBS pH 7.2 or desired final buffer (e.g., in 200 µL buffer resulting in [M] 50 micromolar (µM) labeled aggregates).

16 Sonicate labeled aggregates in a Bioruptor sonication bath (Diagenode) (5 cycles of 5 seconds on – 5 seconds off), or similar.

17 Aliquot, flash-freeze in liquid nitrogen and store at -80 °C.

Note

This protocol can be used for other aggregates like Tau or α-Synuclein aggregates.