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# N_terminal protein labeling

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

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## Abstract

This protocol details how to efficiently label a protein at the N-terminus using Clusterin protein as example.

## Attachments

DOCX

973-2530.docx

19KB

## Materials

### Buffers

- Labeling buffer: [M] 0.1 Molarity (M) sodium bicarbonate buffer pH 8.3
- 1x PBS pH 7.2

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

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

| Equipment | |
|---|---|
| Nap-5 column | NAME |
| Cytiva NAP™ | BRAND |
| 45-000-151 | SKU |
| https://www.fishersci.com/shop/products/illustra-nap-columns-nap-5-2/45000151 | LINK |

## Troubleshooting

## N-terminal protein labeling

1h 30m

1 Exchange the protein buffer to Labeling buffer using a Nap-5 column (Thermo Fisher Scientific, 45-000-151). Equilibrate the column with 10 column volumes (CV: 1 mL). Load the protein onto the column and elute the protein with the corresponding amount of Labeling buffer following the column manufacturer's instructions. Collect each eluted drop into an Eppendorf low binding tube. Measure the protein concentration in each fraction by nanodrop and pool the protein-containing fractions.

Note

The protein will be diluted and partially lost in the process. Therefore, it is recommended to start with relatively high protein concentration. Estimated labeled Clusterin yield: around 300 µL at [M] 30 micromolar (µM) labeled Clusterin if starting with 100 µL purified Clusterin at [M] 200 micromolar (µM) .

2 Dissolve the dye (Alexa488 NHS ester(refer materials section); pHrodo Red Succinimidylester in DMSO. With a pipette tip gently touch the dye powder which will stick to the tip. Immerse the tip in some DMSO previously dispensed in a tube. 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 488 nm 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.

3 Quantify the diluted dye concentration by nanodrop. Dilute the dye with water for measurement in order to reach an absorbance of $\lambda<1$ 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: Absorbance maximum (λmax): 560nm; 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.

4 Add the corresponding amount of diluted dye to the eluted protein to reach a final protein:dye ratio of 1:4-1:10.

5 Incubate 01:30:00 at Room temperature in the dark. 1h 30m

6 Remove free dye by using a Nap-5 column, pre-equilibrated with 1x PBS pH 7.2 buffer or desired final buffer. Collect each eluted drop in an Eppendorf low binding tube. Measure protein concentration in each fraction by nanodrop and pool the fractions containing protein.

Note

Avoid pooling the very last fractions because they may contain free dye.

7 Measure labeling efficiency (dye molarity/protein molarity) by nanodrop. Quantify independently dye concentration and protein concentration.

Note

If labeling Clusterin, it should be noted that it is a heterodimer containing 2 N-termini and therefore the labeling efficiency may be higher than 1.