Quick Guide: Labelling with Alexa Fluor™ 488 NHS Ester

Apparatus and Materials:

- Zeba™ Desalting Chromatography Cartridges, 7K MWCO, 1.0 mL
  ThermoFisher Scientific, catalog No. 89934
- Anhydrous dimethylsulfoxide (DMSO)
- Reaction Buffer - 0.2 M $NaHCO_3$ at pH 8.2 (pH adjusted with HCl), filter sterilized (0.22 µm)
- Alexa Fluor™ 488 NHS ester fluorescent dye
  ThermoFisher Scientific, catalog No. A20100
- PBS buffer - 10 mM phosphate buffer at pH 7.4, 2.7 mM KCl and 137 mM NaCl
  Sigma-Aldrich P4417-100TAB
- UV-VIS spectrophotometer (Nanodrop)
- Pipette and tips - 1 mL, 200 µL, and 10 µL
- Eppendorf tubes®

Method:

1. Prepare 100 µL of a 900 nM solution of Alexa Fluor™ 488 NHS ester dye in reaction buffer. This can be made fresh or diluted down from a stock solution disolved in anhydrous DMSO.

   Note: Once dye is in reaction buffer it must be used quickly. Make sure DMSO contributes less than 4% to the final Volume.

2. Make up 100 µL of a 300 nM solution of protein in reaction buffer by buffer exchange, dilution or direct reconstitution.

   Note: Make sure there are no primary amines (e.g., free amino acids, Tris, urea, etc.) in the protein solution if diluting from an exisiting stock.

3. Directly add all 100 µL of the 900 nM dye solution to the protein solution in a stepwise manner (20 µL at a time) mixing slowly by pipetting up and down after each step (do not vortex).

4. Incubate the reaction mixture at 4 °C overnight or at room temperature for 1 hour (depending on the stability of the protein).

5. Remove the dye using Zeba™ Desalting Chromatography Cartridges, 7K MWCO, 1 mL or similar desalting columns.

5a. Equilibrate the desalting column with 5 column volumes (CV) of reaction buffer.

5b. Load the incubated labelling mixture onto the column.

5c. Elute sample with 1 mL of reaction buffer.

5d. For elution, collect 10 x 100 µL fractions into clean 1.5 mL Eppendorf tubes.

6. To determine labelling efficiency and purity; use a UV-VIS spectrophotometer and measure the absorbance of each fraction to determine which fractions contain the labelled protein without any free dye. For example, a Nanodrop can be used to determine labelling efficiency using the “Proteins & Labels” dialog.

7. Alternatively, use the Fluidity One-W to test the purity of the sample where the approximate molecular weight of the protein is known. If the results given are ~30% lower than expected there is likely to be excess free dye in the sample. Repeat purification until the results given by the Fluidity One-W do not change between purifications.

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