Conjugation Protocol for Amine-Reactive Dyes

In Brief

Succinimidyl esters (NHS esters) are amine-reactive reagents that can be conjugated to (non-protonated) aliphatic amine groups. The primary reactive species for protein amine-conjugation are the ε-amino groups of lysine residues. To avoid protonating these groups

it is important to perform the reaction at a slightly basic pH. In addition, buffers containing primary amines should be avoided, since they will compete for conjugation with the NHS ester.

Please note that NHS esters can be moisture sensitive, so handle accordingly. Where possible, handle and store fluorescent dyes in the dark.

Note: A typical molar ratio for labeling Janelia Fluor®, SE dye:protein is 15:1, however this should be optimized for each specific protein. We recommend trying three different molar ratios of dye:protein to develop a protein-specific protocol for future use.

Reagents

- Prepare a 10 mM stock solution of NHS ester dye in anhydrous DMSO or DMF. Briefly vortex.
- Prepare protein/antibody to be conjugated at approximately 3.0 mg/mL (minimum recommended protein concentration is 2.0 mg/mL†) in either sodium borate (50 mM, pH 8.5) or carbonate buffer (100 mM, pH 8-8.5). N.B. for carbonate buffer, we recommend adding 75 mg/mL sodium bicarbonate solution as indicated below to adjust the pH of the starting protein/antibody PBS solution to approximately 8.25. Protein/Antibody Volume (µL) x 0.112 = Volume sodium bicarbonate required (µL).

† N.B. This is critical for achieving the optimal degrees of labeling (DOLs) for Janelia Fluor® dyes.

Conjugation

Add dye solution to protein solution at the appropriate molar ratio (see note above) while stirring/gently vortexing.

Incubate at room temperature for 60 minutes in the dark†.

Quench the reaction by adding Tris-HCl or Glycine (pH 7.4, 50-100 mM final concentration), incubate with stirring for 10-15 minutes at room temperature (this step is optional).

† N.B. Increasing the incubation time to 18 hours in the dark can increase the DOL with Janelia Fluor® dyes.

Purification

- Remove excess dye with a Zeba™ Spin desalting column (Thermo), with appropriate MWCO, or a PD MiniTrap™ G-25 (GE Healthcare), following the manufacturer's instructions.

Degree of Labeling Calculation

- Dilute the protein-dye conjugate to approximately 0.1 mg/mL and measure the absorbance at 280 nm (protein A_{280}) and at the maximum absorbance wavelength (A_{max}) for the fluorescent dye used (please refer to the individual product descriptions for the max. λabs values).
- Calculate the corrected A_{280} (A_{280C}) using the following equation:

  $$A_{280c} = A_{280} - (A_{max} \times CF)$$

  Please refer to the individual product descriptions for the correction factor (CF) values for the fluorescent dye used.
- Calculate the final protein concentration ([protein] in mg/mL) using the corrected A_{280C} value calculated above, the extinction coefficient (ε) for your protein and the Beer-Lambert Law equation:

  $$A_{280c} = \varepsilon \times [protein] \times l$$
- Calculate the final F:P ratio (Degree of Labeling), where ε_{dye} is the extinction coefficient for the fluorescent dye used, (please refer to the individual product descriptions for this value).

$$F:P = \frac{A_{max} \times MW_{protein}}{[protein] \times \varepsilon_{dye}}$$