Conjugation Protocol for Thiol-Reactive (maleimide) dyes

In Brief

Maleimides are thiol-reactive reagents that can be conjugated to sulfhydryl (thiol) groups. The primary reactive species for protein thiol-conjugation are the sulfhydryl groups of cysteine residues. Cysteine residues can form disulfide bridges via oxidative dimerization which helps stabilize protein ternary structures. Since disulfides do not react with maleimides, it may be necessary to reduce these disulfide bonds prior to conjugation. Conjugation is also reversible, so it is imperative that the buffer used is made and maintained between pH 7–7.5.

Please note that thiols can be oxygen sensitive, so handle accordingly. Where possible, fluorescent dyes should be handled and stored in the dark.

Note: A typical molar ratio for labeling maleimide:protein is between 10:1-20:1, 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

Solutions can be degassed sufficiently by applying a vacuum for several minutes and/or by bubbling through an inert gas such as nitrogen or argon.

- Prepare a 10 mM stock solution of maleimide dye in anhydrous DMSO or DMF. Briefly vortex. *Unused stock solution can be stored in the dark at –20 °C for up to a month.*
- Prepare protein to be conjugated between 1–10 mg/mL in degassed pH 7–7.5 buffer such as 1× PBS, 10–100 mM Tris or 10–100 mM HEPES (other buffers can be used presuming they don't contain thiols).
- Optional for reduction of disulfide bonds: Add 10–100× excess of TCEP (tris-carboxyethylphosphine) to the protein solution, flush with inert gas and close the vial. Keep the mixture for 20–30 minutes at room temperature. DTT (dithiothreitol) can be used instead of TCEP if preferred, but excess DTT should be removed by dialysis prior to conjugation.

Conjugation

- Add dye solution to protein solution at an appropriate molar ratio (between 10–20× molar excess of dye is recommended as a start point) while stirring/gently vortexing.
- Flush vial with inert gas and close tightly.
- Protect from light and incubate either at room temperature for 2 hours or overnight at 2–8 °C.

Purification

- Excess dye can be removed from the conjugate with a gel filtration vial such as a Zeba™ Spin desalting column (Thermo), with appropriate MWCO, or a PD MiniTrap™ G-25 (GE Healthcare), following the manufacturer's instructions.
- Depending on the protein being labeled, dialysis, HPLC, FPLC or electrophoresis may be a preferred purification method.

Storage

- For best results, we recommend immediate use of the purified conjugate solution. If necessary, the conjugate solution can be stored protected from light at 2–8 °C for up to 1 week.
- For longer term storage 5–10 mg/mL BSA and 0.01–0.03% sodium azide should be added to prevent denaturization and microbial growth. If necessary, 50% glycerol can be added and the solution stored can be stored protected from light at –20 °C for up to a year.

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).

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