Conjugation Protocol for Thiol Reactive CoraFluor™ Reagents

In Brief

Thiol reactive CoraFluor™ reagents contain maleimides which 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 maleimides can be moisture sensitive, so handle accordingly. Where possible, handle and store CoraFluor reagents in the dark.

Conjugation Protocol

1. Prepare an aliquot of protein, such as an antibody or nanobody, at a concentration of ≥1 mg mL^{-1} in reaction buffer (e.g. 10-100 mM phosphate buffer, pH 7-7.5) using a 0.5 mL, 7 kDa molecular weight cutoff (MWCO) Zeba™ spin desalting column (Thermo Fisher 89882) according to the manufacturer's protocol.

2. Add the protein solution to the thiol reactive CoraFluor™ to achieve a molar ratio of ~5-10× CoraFluor™ to protein.

   If performing multiple conjugations, reconstitute CoraFluor™ in 2.5 mM dry DMSO or DMAc and perform conjugation reactions with a final DMSO or DMAc content <10%.

   The molar equivalents of CoraFluor™ can be adjusted accordingly depending on size of the protein and desired degree of labeling.

3. Briefly vortex the reaction mixture and allow to stand at room temperature for 1 h.

4. Remove unreacted maleimide complex by buffer exchange into desired storage buffer (e.g. 50 mM sodium phosphate buffer, pH 7.4, with 150 mM NaCl and 0.05% (vol/vol) TWEEN®- 20) using a 0.5 mL, 7 kDa MWCO Zeba™ spin desalting column, according to the manufacturer's instructions.

5. Determine concentration and degree of labeling (DOL) using the below calculations.

Degree of Labeling Calculation

1. Determine the corrected absorbance at 280 nm value ($A_{280,corr}$) of the antibody/nanobody/protein conjugate by measuring A_{280} and A_{340} using:

   $$A_{280.corr} = A_{280} - (A_{340} \times c.f)$$

   c.f. is the correction factor for the terbium complex contribution to A_{280} and is equal to 0.157.

2. Determine the concentration of antibody/protein/nanobody conjugate, c_{ab} (in M) using:

   $$c_{ab} = \frac{A_{280.corr}}{\varepsilon_{ab}} \times b$$

   where ε_{ab} is the antibody/protein/nanobody extinction coefficient at A_{280} and b is the path length in centimeters.

3. Determine the concentration of covalently bound terbium complex, c_{Tb} (in M) using:

   $$C_{Tb} = \frac{A_{340}}{\varepsilon_{Tb}} \times b$$

   where ε_{Tb} is the complex extinction coefficient at A_{340}, equal to 22,000 M^{-1} cm^{-1}, and b is the path length in centimeters.

4. Calculate the degree of labeling (DOL) using:

   $$DOL = \frac{c_{TB}}{c_{ab}} \times b$$

References

1. Payne, N.C., Kalyakina, A.S., Singh, K. *et al.* Bright and stable luminescent probes for target engagement profiling in live cells. Nat Chem Biol **17**, 1168–1177 (2021).