Conjugation Protocol for Amine Reactive CoraFluor Reagents

In Brief

Amine reactive CoraFluor reagents contain pentafluorophenyl esters (pfp esters) which 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 pfp ester.

Please note that pfp esters can be moisture sensitive, so handle accordingly. Where possible, handle and store CoraFluor reagents in the dark.

Conjugation Protocol

1. Prepare a 100 µL aliquot of an antibody, protein or nanobody at a concentration of ≥1 mg/mL in reaction buffer (100 mM sodium carbonate buffer, pH 8.5 + 0.05% (vol/vol) TWEEN®-20) 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 amine reactive CoraFluor (2.5 mM in dimethylacetamide, DMAc, or dry DMSO) to the antibody/protein/nanobody solution to achieve a molar ratio of ~12–15× CoraFluor to antibody or 4–5× to nanobody (final DMAc or DMSO content < 5%).

   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 organic solvent and unreacted pfp ester complex by buffer exchange into storage buffer (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 protocol.

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

6. Dilute antibody/protein/nanobody conjugates to the desired concentration with 50% glycerol then freeze in liquid nitrogen and store at -80 °C.

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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:

$$DOL = \frac{c_{Tb}}{c_{ab}}$$

References

Payne *et al* (2021) Bright and stable luminescent probes for target engagement profiling in live cells. Nat.Chem.Biol.***17*** 1168 PMID:34675420.