

Maleimide Labeling of Proteins and Other Thiolated Biomolecules

The reaction of thiols with maleimides is a process widely used for the bioconjugation and labeling biomolecules, including proteins and peptides. It proceeds according to the following scheme:



Maleimides are electrophilic compounds that show high selectivity towards thiols. While maleimides hardly ever occur in nature, thiols are very abundant. They are encountered in proteins and peptides as cysteine residues. Although natural DNA does not contain thiols, synthetic oligonucleotides with thiol groups can be easily prepared.

Thiols are prone to oxidative dimerization with the formation of disulfide bonds. Cysteine residues thus form disulfide bridges, which stabilize protein tertiary structures. Disulfides do not react with maleimides. Therefore, it is necessary to reduce disulfides before the conjugation and exclude oxygen from the reaction.

Conjugation protocol depends on the solubility of the starting components. For compounds with low aqueous solubility, like most fluorescent dye maleimides, using of an organic co-solvent, such as [DMSO](#) or [DMF](#), is essential.

We recommend the following protocol for conjugation [Lumiprobe dye maleimides](#) with proteins, peptides, and other thiolated biomolecules.

1. Dissolve the protein or other molecule containing thiol to be labeled in a degassed buffer, pH 7-7.5 (PBS, Tris, and HEPES are good, although other buffers containing no thiols can be used) in a plastic vial. Buffer can be degassed by applying vacuum on it for several minutes or by bubbling through inert gas (nitrogen, argon, or helium). For proteins, good concentration is between 1-10 mg/mL.
2. Add an excess of TCEP (tris-carboxyethylphosphine) reagent to reduce disulfide bonds, flush with inert gas, and close. 100× molar excess of TCEP is fine. Keep the mixture for 20 min at room temperature.
3. Dissolve dye maleimide in DMSO or fresh DMF (1-10 mg in 100 μ L).
4. Add dye maleimide solution to thiol solution (20× fold excess of dye), flush the vial with inert gas, and close tightly.
5. Mix thoroughly and keep overnight at 4 °C or room temperature.
6. Purify the product by gel filtration, HPLC, FPLC, or electrophoresis.

For maleimides with poor aqueous solubility, like most dye maleimides, we recommend the use of co-solvent (DMF or DMSO). Maleimides with good aqueous solubility (like sulfo-Cyanine maleimides) can be dissolved in water. If precipitation occurs, increase the content of organic co-solvent in the mixture to achieve better labeling.

Dialysis is recommended as a means of purification only for maleimides with good aqueous solubility.

