We recommend using the following general protocol for the labeling of proteins, peptides, and other thiolated biomolecules with APExBIO dye maleimides.

1. Dissolve the protein or other molecule containing thiol to be labeled in degassed buffer (PBS, Tris, HEPES are good, although others buffers containing no thiols can be used) at pH 7-7.5 in 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. 100x molar excess of TCEP is fine. Keep the mixture for 20 minutes at room temperature.

3. Dissolve maleimide in DMSO or fresh DMF (1-10 mg in 100 uL).

4. Add dye solution to thiol solution (20x fold excess of dye), flush vial with inert gas, and close tightly.

5. Mix thoroughly, and keep overnight at room temperature, or 4 Celsius.

6. Purify by gel filteration, HPLC, FPLC, or electrophoresis.

For maleimides with poor aqueous solubility, like most dye maleimides, we recommend use of co-solvent (DMF or DMSO). Maleimides with good aqueous solubility (like sulfo-Cy maleimides) can be dissolved in water. If precipitation occurs, increase 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.