Protocol for Protein Modification with PTAD:

Part 1: PTAD activation

• Mix together 1:0.98 molar equivalents of unactivated PTAD to 1,3-dibromo-5,5-dimethylhydantoin (product CP-7012) in organic solvent (preferred solvents are DMF or acetonitrile, avoid using DMSO)

• Color change is observed from colorless/pale yellow to deep red (approximately 5 min of mixing).

• After the solution turns red, store the now activated reagent on ice and use for protein modification within 30 min.

Part 2: Protein modification

• Add protein solution in mixed phosphate/Tris buffer or Tris buffer (pH should be 6 - 9) to the eppendorf tube (or other vial) containing the activated PTAD reagent prepared above and mix gently at room temperature for up to 30 min. Preferably use 10-fold molar excess of reagent relative to protein. Use protein at a minimum concentration of 1 mg/ml (higher concentrations are preferred for enhanced labeling).

• Remove excess unreacted PTAD by gel filtration.