General Procedures for Ferrocene NHS Ester Conjugation

Peptide General Conjugation Protocol

Suspend the peptide (or protein) into a non-amine buffer at pH 8.3 (for example 10 mM Hepes, 150 mM NaCl, pH 8.3) to the highest concentration that the peptide maintains solubility. Dissolve Ferrocene NHS Ester in DMSO at 100mg/ml, then immediately dispense this solution into the peptide-buffer solution at a 100-1000X molar excess of Ferrocene Ester relative to peptide. Let the reaction proceed at RT with gentle shaking for 4 hrs. (If you are conjugating a protein in a cell lysate, use non-amine protease inhibitors and perform the reaction overnight at 4^{o}C with gentle shaking). Purify the protein-ferrocene conjugate using gel filtration, or desalt in a spin column, collecting the ferrocene-peptide in the void volume. The Ferrocene-linked peptide will attain a yellowish coloration. The peptide-ferrocene conjugate can be followed at 438 nm in correspondence with the peptide bond absorbance peak.

Oligonucleotide Conjugation Protocol

Ferrocene-NHS Ester (9.85 mg, 30.11 micromol) is dissolved in 1.0 milliliter of methyl sulfoxide and 3 micromole amino modified oligonucleotide is dissolved in 800 microliter of 0.2 M sodium carbonate buffer (pH 9.5). The ester solution (400 microliter) is added to the amino-oligonucleotide solution. The mixture is left for 16 hour at 4^{O}C, after which it is chromatographed on a Sephadex G-25 column using de-ionized water/carbonate buffer (50/50) as eluent. The fraction with yellow color is dialyzed against water to remove excess salts and unreacted reagents, and then freeze-dried. The final product is stored in the refrigerator until use.