General Procedures for Malachite Green and Leuco Malachite Green NHS Ester Conjugation

Peptide General Conjugation Protocol

Suspend the peptide 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 remains soluble. Dissolve Malachite Green NHS Ester in DMSO at 100mg/ml, then immediately dispense into the peptide-buffer solution at a 100-1000X molar excess of Malachite Green NHS Ester relative to peptide. Assume a 30% purity of the technical grade Malachite-Green NHS Ester starting material, therefore multiply the required moles of Malachite-Green NHS Ester by x3 to correct the molar stoichiometry. Let the reaction proceed at RT with gentle shaking for 4 hrs. If you are conjugating malachite green to a protein in a cell lysate, add non-amine protease inhibitors and perform the reaction overnight at 4^{o}C with gentle shaking. The peptide-malachite green conjugate can be purified using gel filtration, or desalted in a spin column, by collecting the malachite green labeled peptide in the void volume. The malachite green-linked peptide should attain a green-blue coloration. The malachite-green labeled peptide can be followed at 616 nm in correspondence with the peptide bond UV absorbance peak. The final product is stored at -20^{o}C.

Oligonucleotide Conjugation Protocol

Malachite Green NHS Ester (30 mg, 90 micromole) 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 8.5). 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 green-blue coloration with linked oligonucleotide 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.